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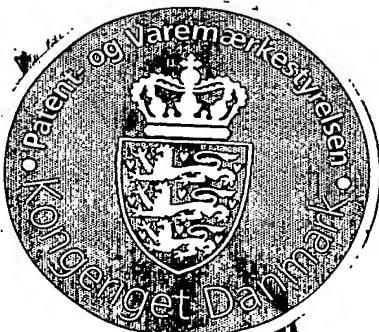
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Title: Compounds comprising LPA

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Patent- og Varemærkestyrelsen
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Field of invention

- 5 The present invention relates to new peptide compounds capable of low-affinity binding to fibroblast growth factor receptor (FGFR), said compounds comprise at least two amino acid sequences, wherein at least one of these two amino acid sequences is capable of binding to FGFR. The invention discloses FGFR binding amino acid sequences and features pharmaceutical compositions comprising thereof. Invention also relates to uses of the compounds and pharmaceutical compositions for the treatment or prevention of different pathological conditions wherein FGFR function plays a role in pathology and/or recovery from disease. New peptide compounds of the invention are obtainable by the ligand presenting assembly (LPA) method.
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Background of invention

- Brain plasticity and the mechanisms controlling plasticity are central to learning and memory as well as the recovery of function after brain injury. While it is clear that neurotrophic factors are one of the molecular classes that continue to regulate brain plasticity in the adult central nervous system (CNS), less appreciated but equally profound is the role of cell adhesion molecules (CAMs) in plasticity mechanisms such as long term potentiation, preservation of neurons and regeneration. Ironically, however, CAMs can also reorganise the extra-cellular space and cause disturbances that drive the development of brain pathology in conditions such as Alzheimer's disease and multiple sclerosis. Candidate molecules include the amyloid precursor protein, which shares many properties of a classical CAM and beta-amyloid, which can masquerade as a pseudo CAM. Beta-Amyloid serves as a nidus for the formation of senile plaques in Alzheimer's disease and like CAMs provides an environment for organising neurotrophic factors and other CAMs. Inflammatory responses evolve in this environment and can initiate a vicious cycle of perpetuated neuronal damage that is mediated by microglia, complement and other factors (Cotman et al. (1998) Prog Neurobiol. 55:659-69).
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- 30
- 35 Neural cell adhesion molecules (CAMs) of the immunoglobulin superfamily nucleate and maintain groups of cells at key sites during early development and in the adult.

In addition to their adhesive properties, CAMs homophytic and heterophytic interactions can affect intracellular signalling. Their ability to influence developmental events, including cell migration, proliferation, and differentiation may therefore result both from their adhesive as well as their signalling properties.

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The neural cell adhesion molecule, NCAM, was the first discovered neural CAM. Since the discovery NCAM has been intensively studied and now it is well characterised (Ronn et al. (2000) *Int J Dev Neurosci* 18:193-9) NCAM belongs to the immunoglobulin (Ig) superfamily. Its extracellular part consists of five Ig-like and two fibronectin type III (F3) modules. NCAM assists both the cell-cell and cell-substratum interactions. NCAM binds to various extracellular matrix components such as heparin/heparan sulfate, chondroitin sulfate proteoglycans, and different types of collagen. Cell-cell interactions are mostly assisted by the NCAM homophilic interaction. The different modules of NCAM have been shown to perform distinct functions. Thus, NCAM homophilic binding is believed now to depend on the first three Ig modules. The heparin binding sequence is localised to the Ig2 module. NCAM also binds to the neural cell adhesion molecule L1. This interaction is believed to take place between the fourth Ig module of NCAM and an oligomannosidic moiety expressed on L1. The two membrane-proximal F3 modules of NCAM have been shown involved in fibroblast growth factor receptor (FGFR) binding.

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A number of research groups has now accumulated a large body of evidence indicating that intracellular signalling cascades underlying the NCAM-mediated axonal outgrowth are similar to signal transduction cascades which are activated due to stimulation of FGFR (Povlsen et al. (2003) *Neurochem Res* 1:127-41).

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Fibroblast growth factor receptors (FGFRs) are a family of four closely related receptor protein tyrosine kinases consisting extracellularly of three Ig-like modules and intracellularly of a split tyrosine-kinase module (Powers et al. (2000) *Endocr Relat Cancer* 7:165-97). The receptors are known as key regulators of morphogenesis, development, angiogenesis, and wound healing. FGFR activation and signalling are dependent on dimerization of the receptor which is induced by a high affinity binding of its ligand, fibroblast growth factor (FGF), and it also requires participation of cell surface heparin or heparan sulphate proteoglycans. Fibroblast growth receptors play a prominent role in the functioning of the peripheral and central neural system

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(Jungnickel et al, (2004) Mol Cell Neurosci. 25:21-9; Reuss and von Bohlen und Halbach (2003) Cell Tissue Res. 313:139-57).

NCAM has been regarded as a member of a new class of putative alternative ligands of FGFR, and recently there has been obtained evidence for a direct interaction between NCAM and the receptor (Kiselyov et al. (2003) Structure (Camb) 11:691-701). The identified NCAM fragment having the sequence EVVV-VAENQQGKSKA (FGL peptide) involved in the direct interaction between NCAM and FGFR has recently been suggested as a new candidate compound for the treatment of a variety of pathologic disorders where the regulation of activity of FGFR may play the key role (WO 03/016351).

WO 03/016351 describes some biological effects of the FGL peptide due its capability of binding and activating FGFR. According to WO 03/016351 presentation of a single copy (monomer) of the peptide to neuronal cells in vitro is enough to promote cell survival and differentiation. However, a rather high concentration of the peptide is needed to achieve the effects.

Multiple presentation of peptide sequences, e.g. antigenic peptides, has been shown to be a valuable mean to amplify biological responses of peptide sequences in vitro (Berezin and Bock (2004) J Mol Neurosci.22:33-39; Ronn et al (1999) Nat Biotechnol 17:1000-5), and it has also been effectively used in vivo. Thus, Cambon et al. (2004) (J Neurosci. 17:4197-204).

Synthetic dendritic polymers of biologically active peptide sequences are now commonly used type of multiple presentation of peptides in research and some clinical applications. A dendritic polymer consists of a number of copies of a monomeric peptide sequence, which are attached to a core molecule at multiple sites forming thereby a branching polymer. Lysine is most commonly used amino acid in the core matrix because it has two reactive amino groups available for branching reactions. The multimerization of the peptide sequences on the lysine core is known as the multiple antigen peptide (MAP) presentation. A method for the synthesis of MAP(s) (dendrimeric peptides) was described in PCT/US90/02039.

In general, two routs can be used for the synthesis of a dendrimeric peptide, namely the direct and indirect route. In both cases, the C-terminal core is first assembled on

a solid support using bi-protected Boc-Lys(Boc) in case of Boc chemistry, or Fmoc-Lys(Fmoc) in the case of Fmoc chemistry in order to obtain the desired degree of branching.

- 5 In the direct route synthesis is done by stepwise assembling of the particular peptide on the lysinyl core by the well-known Merrifield method. This stepwise method produces peptides with a C to N orientation. The peptide chains may be synthesised in tandem, or, alternatively, each peptide can be synthesised on the different lysine arms of the core using orthogonal deprotection methods.

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Although this approach for dendrimeric peptide synthesis is convenient, the quality of the final product may be questionable and problems of obtaining a well-defined product have been widely discussed in the literature. Problems arise during chain assembly. The MAP type dendrimers are macromolecules, where each single pep-

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ptide chains may uncontrolled interact with each other, preventing thereby high efficiency coupling to the core and leading to production of non-homogenous compound, which require effort to purification. Furthermore, the characterisation of these products by, e.g. electrospray mass spectroscopy (ES-MS) is difficult and as a result, dendritic peptide products are often used without full characterisation.

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In the indirect route the synthesis is done by coupling of purified unprotected peptide fragments to the core matrix. Two general methods for this type of ligation may be used, which, respectively, are based on thiol and carbonyl chemistries.

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The thiol chemistry may be carried out by incorporation of chloroacetyl group(s) on the lysine matrix and subsequent coupling to a purified, synthetic N-terminal cysteinyl peptide to yield a dendrimer with unambiguous structure. The reverse placement may be achieved with thiol on the core matrix by using an S-acetyl group on the lysinyl core and the haloacetyl group on the peptide. In the carbonyl chemistry

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the condensation takes place between a carbonyl group and a weak base. There are two types of weak bases, which may be used for this reaction. The first includes hydroxylamines and hydrazines, and the second includes 1,2-di-substituted moieties such as 1,2-aminoethanethiol. The last two groups are found in N-terminal Cys and The, respectively, and the condensation products are thiazolidine and oxazolidine.

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The carbonyl group on the core matrix may be obtained by periodate oxidation of N-

terminal Ser or Cys. Both methods are well known in the art (Lu et al., (1991) Mol Immunol 28:623-630; Defoort et al., (1992) Int J Pept Prot Res 40:214-221; Drijfhout et al. (1991) Int J Pept Prot Res 37:27-32).

- 5 Another problem associated with the MAP type compounds is orientation of peptide chains. In the direct route with stepwise assembling of the desired peptide on the lysinyl core only a compound having peptide chains with C to N orientation can only be obtained. The indirect route allows the both orientations, C to N and N to C, but the process has other major disadvantages, e. g. undesirable side reactions due to
10 oxidation of disulphides, leading to impure products, interference with cystein residues present in the native peptide chains, the procedure demands optimisation for every synthesis.

- 15 Finding the optimal number of multiplication for a peptide sequence is another problem to be solved. Most studies of dendritic peptide polymers synthesised by the MAP method have been carried out using tetra- or octameric polymers. However, application of the compounds comprising 4-8 sequences of 15 and more amino acids in vivo is challenged by a problem of capability of a compound to penetrate the blood-brain barrier, which is crucial if the compound is for the treatment of the brain.
20 Dimers of the peptide sequences could be a solution to this problem, in case the dimers possess activity of the dendritic tetramer analogues. Production of peptide dimers is described in the art (WO00/18791).

- 25 Although, a dendrimeric version of the FGL peptide consisting of four sequences of FGL has been shown to be effective in vivo in rat (Cambon et al. (2004) J Neurosci. 17:4197-204), it is problematic to use the dendrimer as a drug in treatment of human patients. HPLC purification of the dendritic tetramer of FGL peptide revealed that the final product of the synthesis is heterogenous and characterisation of the product is problematic. An attempt to solve this problem by reducing the number of peptide sequences in the compound to two led the authours of the present invention to
30 production of biologically inactive compound.

Summary of invention

A dimer produced by the ligand presenting assembly method, which was described in WO00/18791, surprisingly had biological activity equal to the FGL dendrimer, whereas other types of the FGL dimers did not have biological activity at all.

- 5 It was surprisingly found by the authors of the present invention, that a dimer of a peptide sequence obtainable by the ligand presenting assembly (LPA) method has biological activity of a dendrimer comprising 4 copies of the sequence. Accordingly, the first aspect the present invention is concerned a compound comprising at least two peptide sequences, wherein each of said two sequences individually comprising
10 an amino acid sequence of the formula

L1-A-L2-B-L3-C-L4-D-L5

wherein

- one of A, B, C, D is selected from a hydrophobic amino acid residue,
15 one of A, B, C, D is selected from a basic amino acid residue or Ser, Thr, Asn or Gln,
one of A, B, C, D is selected from an acidic amino acid residue or Ser, Thr, Asn or Gln,
one of A, B, C, D is Gly or Ala, and
20 L1, L2, L3, L4 and L5 is selected from a chemical bond or an amino acid sequence having n amino acid residues, wherein n is an integer of from 0 to 5,
wherein said peptide sequences being connected to each other through the linker having the general formula

- 25 X[(A)nCOOH][(B)mCOOH]

n and m independently are an integer of from 1 to 20,

X is HN, H₂N(CR₂)pCR, RHN(CR₂)pCR, HO(CR₂)pCR, HS(CR₂)pCR, halogen-(CR₂)pCR, HOOC(CR₂)pCR, ROOC(CR₂)pCR, HCO(CR₂)pCR, RCO(CR₂)pCR,

- 30 [HOOC(A)n][HOOC(B)m]CR(CR₂)pCR, H₂N(CR₂)p, RHN(CR₂)p, HO(CR₂)p, HS(CR₂)p, halogen-(CR₂)p, HOOC(CR₂)p, ROOC(CR₂)p, HCO(CR₂)p, RCO(CR₂)p, or [HOOC(A)n][HOOC(B)m](CR₂)p, wherein p is 0 or integer of from 1 to 20,

A and B independently are a substituted or unsubstituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety, a substituted

- 35 or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety,

or A and B together form a substituted or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

5 In another aspect, the invention relates to a pharmaceutical composition comprising a compound as defined above.

In still another aspect, the invention concerns using the above compound for the manufacture of a medicament for treatment of

- a) conditions of the central and peripheral nervous system associated with post-operative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression; for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel;
- b) postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression;
- c) promotion of wound-healing;
- d) prevention of death of heart muscle cells, such as after acute myocardial infarction, or after angiogenesis;
- e) promotion of revascularisation;

- f) stimulation of the ability to learn and/or the short and/or long-term memory;
- 5 g) prevention of cell death due to ischemia;
- h) prevention of body damages due to alcohol consumption;
- i) treatment of prion diseases;
- 10 j) treatment of cancer.

Description of Drawings

Figure 1 shows a flow chart of the synthesis of the LPA-type FGL dimer (FGL_L)

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Figure 2 presents the HPLC purification profile of FGL_L

Figure 3 presents the HPLC purification profile of FGL_D

20 Figure 4 demonstrates the effect of the FGL peptide on survival of primary neurons treated with various neurotoxic agents.

Dopaminergic neurons (DN) (a and b) from day 15 rat embryos grown at a density of 150,000 cells/cm² for six days without or with various concentrations of peptide on 24-well cell culture plates coated with poly-D-lysine were exposed to 100 µM 6-OHDA for two hours. Medium was changed and various concentrations of FGL_d were added. The neurons were grown for another 24 hours before the cultures they were fixed and immunostained for tyrosine hydroxylase.

25 Hippocampal neurons (HN) (c and d) from day 19 rat embryos were seeded at a density of 40,000 cells/cm² on poly-L-lysine coated 8-well permanox chamber slides and grown for 24 hours in medium containing 20 µM Amyloid-β 25-35 peptide (Aβ 30 25-35) at the presence of various concentrations of FGL_d , before they were fixed and stained with Hoechst 33258.

Cerebella granular neurons (CGN) (e and f) from postnatal day 7 rats were grown at a density of 100,000 cells/cm² for 7 days on poly-L-lysine coated microtiter plates in the medium containing 40 mM KCl, then the medium was substituted to a 5 mM

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KCl containing medium supplemented with various concentrations of FGL_d. After two days of incubation, the cultures were fixed and stained with Hoechst 33258.

- (a) - Effect of 10 ng/ml GDNF on survival of dopaminergic neurons treated with 6-OHDA.
- 5 (b) Effect of various concentrations of FGL_d on survival of dopaminergic neurons treated with 6-OHDA.
- (c) Effect of 50 ng/ml BDNF on Aβ 25-35-treated hippocampal cultures.
- (d) Effect of various concentrations of FGL_d on survival of hippocampal neurons treated with Aβ 25-35.
- 10 (e) Effect of 50 ng/ml IGF-1 on CGN cultures induced to undergo apoptosis by depriving the neurons of high potassium.
- (f) Effect of various concentrations of FGLd on CGN cultures induced to undergo apoptosis.

Results from at least four independent experiments for each type of culture are expressed as percentage ± SEM of live neurons as compared to the total number of neurons. Control cultures induced to undergo cell death without FGL-treatment were set at 100 %. + p<0.05, ++ p<0.01 when compared to the untreated controls. * p<0.05, ** p<0.01 and *** p<0.001 when compared to the cultures induced to undergo cell death.

20 **Figure 5** demonstrates the effect of the FGL peptide on DNA-fragmentation in CGN cultures induced to undergo apoptosis by changing the medium after the neurons were grown for six days in high KCl medium (40 mM) to a low KCl medium (5 mM KCl). The number of neurons with fragmented DNA was measured using the ApoAlert apoptosis detection kit and the fraction of TUNEL-positive neurons was estimated by counting. Results from at least four independent experiments are shown as percentage of cells showing DNA-fragmentation relative to the total number of cells ± SEM with control cultures in low KCl set at 100 %.

- 25 (a) Effect of depriving CGN cultures of high KCl on number of cells undergoing apoptosis.
- (b) Effect of FGLd in apoptosis-induced CGN cultures.

* p<0.05, ** p<0.01 and *** p<0.001 when compared to low KCl-treated CGN cultures.

Figure 6 shows the effect of FGL on phosphorylation of Erk1/2. CGN were grown for three days at a density of 290,000 cells/cm² on poly-L-lysine coated microtiter plates, were subsequently treated with FGL_d for 10 - 90 min, fixed and stained with antibodies against phospho-p42/44. The total number of neurons was estimated using crystal violet staining. Results from at least three independent experiments are expressed as percentage ± SEM with untreated cultures set at 100 %. * p<0.05 and ** p<0.01 when compared to the unstimulated controls.

Figure 7 shows the effect of FGL on phosphorylation of Akt. CGN were grown for three days at a density of 290,000 cells/cm² on poly-L-lysine coated microtiter plates, were subsequently treated with FGL_L at various concentrations for 10 or 30 minutes respectively and subsequently fixed and stained with antibodies against Akt phosphorylated on Ser473. The total number of neurons was estimated using crystal violet staining. Results from at least three independent experiments are expressed as percentage ± SEM with untreated cultures set at 100 %. * p<0.05 when compared to the unstimulated controls.

Figure 8 shows the effect of inhibitors of FGFR, MEK and PI3K on FGL-induced survival of CGN induced to undergo apoptosis. CGN were grown at a density of 100,000 cells/cm² for seven days on poly-L-lysine coated 8-well permanox slides in medium containing 40 mM KCl, before the medium was changed to a 5 mM KCl containing medium together with FGL_d. Thereafter the MEK inhibitor PD98059 (●), PI3K inhibitor LY294002 (▲) and FGFR inhibitor SU5402 (■) were added at various concentrations. The addition of the inhibitors followed in all cases by the addition of 10 µg/ml FGL_d. After two days of incubation with the peptide and inhibitors, the cultures were fixed and stained with Hoechst 33258. Results from at least four individual experiments are shown as percentage of the number of live neurons relative to the total number of neurons ± SEM, with control cultures treated with FGL_d set at 100 %.

Figure 9 shows the effect of the FGL peptide on neurite outgrowth from dopaminergic (●), hippocampal (▲) and cerebellar granule neurons (■). Dopaminergic neurons were grown at a density of 100,000 cells/cm² on poly-D-lysine coated 24-well cell culture plates for 72 hours with various concentrations of FGL_d. The cultures were subsequently immunostained for tyrosine hydroxylase. Hippocampal neurons and

CGN were plated at a density of 10,000 cells/cm² on 8-well permanox chamber slides and incubated for 24 hours in the presence of various concentrations of FGL_d. Subsequently the neurons were immunostained for GAP-43. Results from at least five independent experiments for each neuronal culture are shown as percentage ± SEM with the untreated controls set at 100 %. * p<0.05, ** p<0.01, *** p<0.001 when compared to untreated controls.

Figure 10 shows the effect of three dimeric versions of the FGL peptide on neurite outgrowth of hippocampal neurons from newborn rats. Primary cultures of hippocampal neurons were grown for 24 hours at a density of 10,000 cells/cm² with various concentrations of FGL_L (■), FGL_{lys} (▲) or FGL_{cys} (●) and then were immunostained for GAP-43. Results from at least four independent experiments are shown as percentage ± SEM with untreated controls set at 100 %. ** p<0.01 when compared to the untreated controls.

Figure 11 shows the effect of inhibitors of FGFR, MEK and PI3K on FGL-induced neurite outgrowth in CGN cultures. CGN cultures were grown for 24 hours at a density of 10,000 cells/cm² on 8-well permanox slides in the presence of the FGL peptide and an inhibitor. PD98059 (●) was added at concentrations of 12.5, 25 and 50 μM simultaneously with 10 μg/ml FGL_d; LY294002 (▲) was added at concentrations of 3.5, 7 and 10 μM simultaneously with 27 μg/ml FGL_d. SU5402 (■) was added in concentrations of 20, 40 and 80 μM simultaneously with 27 μg/ml FGL_d. After the treatment cells were fixed and immunostained for GAP-43. Results from at least four independent experiments are shown as percentage ± SEM, with FGL_d-treated control cultures set at 100 %. The dashed line indicates the average neurite length in cultures without FGL_d-treatment. * p<0.05, ** p<0.01 when compared to the FGL_d-treated controls.

Figure 12 shows the effect of FGL_d, FGL_{control} and vehicle on the rate of FM 1-43 de-staining of primary cultures of hippocampal neurons from E19 rats. Each value represents the mean of 4-8 experiments and error bars indicate S.E.M. Neither treatment with 5 μg/ml FGL_d for any time period, nor the treatment with 20 μg/ml for 24 hours had any effect on the rate of FM 1-43 unloading. In contrast, synapses in cultures treated with 20 μg/ml FGL_d for 1 hour and 48 hours showed a significantly increased rate of FM 1-43 unloading when compared to control cultures, indicating an enhanced presynaptic re-

sponse in these cultures. The values were compared using an unpaired t-test. * $p=0.04$ (1h) and ** $p = 0.0032$ (48h).

Figure 13 demonstrates the effect of early sub-occipital administration of FGL_L, FGL_D and FGL_{control} on memory (Social Recognition test) of rats in which cognitive impairment was induced by intracerebroventricular (i.c.v.) -injection of the (25-35) β -amyloid fragment. The results are from the experiment in which the suboccipital administration of 5.0 μ g FGL per administration per rat was done at days 7, 10, and 13 after A- β -induced neurotoxicity. The Social Recognition test was performed 21 days after i.c.v. administration of the (25-35) β -amyloid fragment and estimated as the ratio between the times the adult rat spent exploring a juvenile rat during a first (T_1) and a second (T_2) meeting. The number of animals in the groups varied from 4 to 22. The results were analysed using one-way ANOVA ($F(2,27)=15.4$, $P<0.0001$) and significance of results was contingent on achieving a p-value of less than 0.05 (** $p<0.01$), when compared to the A- β /V group (Newman-Keuls post-test). A statistically significant effect was also observed between the control and the V/V-treated group compared to the A- β /V group ($p<0.001$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively. 'V/V' stands for the rats received vehicle as replacement of A- β and FGL.

Figure 14 shows the effect of early suboccipital administration of FGL_L and FGL_D on formation of amyloid burden in the cingulate cortex of rats in which cognitive impairment was induced by i.c.v. -injection of the (25-35) β -amyloid fragment (5.0 μ g FGL per administration per rat) on days 7, 10 and 13 after (25-35) β -amyloid (A- β) fragment-induced neurotoxicity. The number of animals in the groups varied from 3 to 13. Results were analysed using one-way ANOVA ($F(2,18)=5.2$, $p=0.016$), and significance of results was contingent on achieving a p-value of less than 0.05 (* $p<0.05$ and ** $p<0.01$), when compared with the A- β /V group (Newman-Keuls post-test). A statistically significant difference was observed between the V/V treated group and the A- β /V treated group ($p<0.05$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 15 shows the effect of early sub-occipital administration of FGL_L and FGL_D on formation of the amyloid burden in the CA3 area of hippocampus of rats in which

cognitive impairment was induced by i.c.v. -injection of the (25-35) β -amyloid fragment (5.0 μ g FGL per administration per rat) on days 7, 10 and 13 after (25-35) β -amyloid (A- β) fragment-induced neurotoxicity. The number of animals in the groups varied from 2 to 12. Results were analysed using one-way ANOVA ($F(2,15)=9.54$, 5 $p=0.002$), and significance of results was contingent on achieving a p-value of less than 0.05 (** $p<0.001$), when compared with the A- β /V group (Newman-Keuls post-test). A statistically significant difference was observed between the V/V treated group and the A- β /V treated group ($p<0.01$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

10 **Figure 16** shows the effect of early sub-occipital administration of FGL_L and FGL_d on neuronal death in the hippocampus of rats in which cognitive impairment was induced by i.c.v. -injection of the (25-35) β -amyloid fragment. The effect of 5.0 μ g FGL per administration per rat, administered sub-occipitally on days 7, 10 and 13 15 after (25-35) β -amyloid (A- β) fragment-induced toxicity, on neuronal cell death in the CA3 zone of the rat hippocampus. The number of animals in the groups varied from 4 to 8. The results were analysed using one-way ANOVA ($F(3,17)=13.76$, $p<0.001$) and significance of results was contingent on achieving a p-value of less than 0.05 20 (** $p<0.001$), when compared with the A- β /V group (Newman-Keuls post-test). A statistically significant difference was observed between the Control group and the A- β /V treated group ($p<0.001$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

25 **Figure 17** demonstrates the effects of 5.0 μ g FGL_L (per administration per rat) administered sub-occipitally on days 30, 33, and 36 after (25-35) β -amyloid fragment (A- β)-induced neurotoxicity in the Social Recognition test. The Social Recognition test was performed 44 days after i.c.v. administration of the A- β fragment and estimated as the ratio between the times the adult rat spent exploring a juvenile rat during a first (T_1) and a second (T_2) meeting. The number of animals per group varied from 4 to 5. The results were analysed using unpaired t-test and significance of 30 results was contingent on achieving a p-value of less than 0.05 (* $p<0.05$), when compared to the A- β /V group. A significant impairment of short-term memory was induced by treatment with A- β ($p<0.05$, unpaired t-test between Control and A- β /V).

'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 18 shows the effects of 5.0 μg FGL_L (per administration per rat) administered 5 sub-occipitally on days 30, 33 and 36 after A- β fragment-induced neurotoxicity on the amyloid burden in the cingulate cortex of rats. The number of animals per group varied from 3 to 13. The results were analysed using one-way ANOVA ($F(2,12)=18.6$, $P=0.002$) and significance of results was contingent on achieving a p-value of less than 0.05 (** $p<0.001$) when compared with the A- β /V group (Bonferroni's multiple comparison test). A statistically significant difference was observed 10 between the V/V-treated group and the A- β /V-treated group ($p<0.001$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 19 shows the effects of 5.0 μg FGL_L (per administration per rat) administered 15 sub-occipitally on days 30, 33 and 36 after (25-35) β -amyloid (A- β) fragment-induced neurotoxicity on the amyloid burden in the CA3 area of hippocampus of rats. The number of animals per group varied from 3 to 5. The results were analysed using one-way ANOVA ($F(2,9)=10.6$, $P=0.004$) and significance of results was contingent on achieving a p-value of less than 0.05 (** $p<0.001$) when compared with 20 the A- β /V group (Newman-Keuls post-test). A statistically significant difference was observed between the Control-treated group and the A- β /V-treated group ($p<0.01$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 20 shows the effect of 5.0 μg FGL_L (per administration per rat) administered 25 sub-occipitally on days 7, 10 and 13 after (25-35) β -amyloid (A- β) fragment-induced toxicity on neuronal cell death in the cingulate cortex. The number of animals in the groups varied from 4 to 8. The results were analysed using one-way ANOVA ($F(2,9)=26.9$; $p<0.001$, Newmann-Keuls post-test: A- β /V compared with the control 30 group (** $p<0.001$) and compared with the A- β /FGL_L treated group (** $p<0.01$). Significance of results was contingent on achieving a p-value of less than 0.05, when compared with the A- β /V group (Newman-Keuls post-test). A statistically significant difference was observed between the Control-treated group and the A- β /V-treated

group ($p<0.001$). 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 21 shows the effect of three intranasal administrations of vehicle, 200 μg FGL_L, and 400 μg FGL_L per rat, at days 7, 10 and 13 after i.c.v. administration of the (25-35) β -amyloid (A- β) fragment in the Social Recognition test. The social recognition test was performed 21 days after i.c.v. administration of the (25-35) β -amyloid (β -A) fragment and estimated as the ratio between the times the adult rat spent on exploration of a juvenile rat during a first (T_1) and a second (T_2) meeting. Number of animals in the groups varied from 3 to 8. Results are shown as means \pm SEM and analysed by unpaired t-test ($p<0.001$ of control vs A- β /V) and one-way ANOVA ($F(2,19)=5.6$; $p=0.01$; Newmann-Keuls post-test, $p<0.05$ of A- β /V vs A- β /FGL_{L,400}; and $p>0.05$ of A- β /V vs A- β /FGL_{L,200}; whereas $p<0.05$ of A- β /FGL_{L,400} vs A- β /FGL_{L,200}). Significance of results was contingent on achieving a p-value of less than 0.05 (* $p<0.05$) when compared to the A- β /V treated group. 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 22 shows the effect of three intranasal administrations of vehicle, 200 μg FGL_L, and 400 μg FGL_L per rat, at days 7, 10 and 13 after i.c.v. administration of the (25-35) β -amyloid (β -A) on neuronal cell death in the cingulate cortex. The number of animals in the groups varied from 4 to 8. The results were analysed using unpaired t-test (of the control group versus the A- β /V-treated group ($p<0.001$) and one-way ANOVA ($F(2,16)=8.6$; $p=0.003$), with Newman-Keuls post-test ** $p<0.01$ of A- β /V vs A- β /FGL_{L,400} and $p>0.05$ of A- β /V vs A- β /FGL_{L,200}). Significance of results was contingent on achieving a p-value of less than 0.05 (** $p<0.01$), when compared with the A- β /V treated group. 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively.

Figure 23 shows the effect of 5.9 μg FGL_d administration on rats tested for the Rearing Activity in the Open Field test. The peptide was applied sub-occipitally at days 7, 10, and 13 after the (25-35) β -amyloid fragment induced neurotoxicity. The numbers '1', '2', and '3' indicate the periods of measurement: '1' denotes 1-3 min., '2' denotes 8-10 min., and '3' denotes 18-20 min. 'V/V' indicates that the rats received vehicle as replacement for the (25-35) β -amyloid fragment and FGL_d. The

number of animals per group varied from 9 to 11. 'A- β ' and 'V' are the abbreviations of the (25-35) β -amyloid fragment and vehicle, respectively. The results were analysed using unpaired t-test and significance of results was contingent on achieving a p-value of less than 0.05 (*p<0.05, **p<0.01); when compared with the first measurement period (1-3 min.).

5 Figure 24 shows the performance of the Surface Righting Reflex by rat pups at PND4 after intranasal administration of 2.6 μ g FGL_L, FGL_d, or FGL_{control} per pup at PND 1, 2, and 3. The number of litters of each treatment group was six. The results
10 were analysed using one-way ANOVA ($F=4.05$; $P=0.039$), and the Newman-Keuls test showed a statistically significant effect of FGL_L ($P<0.05$) and FGL_d ($P<0.05$) when compared to the control- and FGL_{control} groups.

15 Figure 25 shows the results of the Negative Geotaxis Reflex Performance by rat pups at PND 6 and PND 9 after intranasal administration of 2.6 μ g FGL_L, FGL_d, or FGL_{control} per pup at PND 1, 2, and 3. The number of litters of each treatment group was six. The results were analysed using one-way ANOVA ($F_{PND6}=5.14$; $P_{PND6}=0.008$). At PND 6, statistically significant effects were observed for the FGL_L (*p<0.05) and FGL_d (**p<0.01) groups, when compared to the control- and FGL_c groups. At PND 9, no significant effect of FGL was observed (One-way ANOVA:
20 $F_{PND9}=0.94$; $P_{PND9}=0.44$).

Figure 26 is a schematic presentation of the time course of in vivo studies.

25 **Detailed description of the invention**

1. Compound

30 Compounds capable of modulating the function of fibroblast growth factor receptor (FGFR) are of greatest importance in view of development of effective drugs for therapeutic treatment of a variety of diseases and pathologic conditions. Thus, it is an object of the present invention to provide novel compounds capable of binding to FGFR and modulating FGFR activity. According to the invention the compounds comprise at least two peptide sequences comprising a structural motif common for

amino acid sequences capable of binding FGFR, and a linker that connects said peptide sequences to each other.

1.1 Peptide sequences

- 5 Thus, in one aspect the invention concerns at least two peptide sequences, wherein each of said sequences individually comprising the amino acid sequence of the formula

L1-A-L2-B-L3-C-L4-D-L5

- 10 wherein
one of A, B, C, D is selected from a hydrophobic amino acid residue,
one of A, B, C, D is selected from a basic amino acid residue or Ser, Thr, Asn or Gln,
one of A, B, C, D is selected from an acidic amino acid residue or Ser, Thr, Asn
15 or Gln,
one of A, B, C, D is Gly or Ala, and
L1, L2, L3, L4 and L5 is selected from a chemical bond or an amino acid se-
quence having n amino acid residues, wherein n is an integer of from 0 to 5.

- 20 In the present context the standard one-letter code for amino acid residues as well as the standard three-letter code are applied. Abbreviations for amino acids are in accordance with the recommendations in the IUPAC-IUB Joint Commission on Bio-
chemical Nomenclature Eur. J. Biochem, 1984, vol. 184, pp 9-37. Throughout the
25 description and claims either the three letter code or the one letter code for natural amino acids are used. Where the L or D form has not been specified it is to be un-
derstood that the amino acid in question has the natural L form, cf. Pure & Appl.
Chem. Vol. (56(5) pp 595-624 (1984) or the D form, so that the peptides formed may
be constituted of amino acids of L form, D form, or a sequence of mixed L forms and
30 D forms.

- Where nothing is specified it is to be understood that the C-terminal amino acid of a peptide of the invention exists as the free carboxylic acid, this may also be specified as "-OH". However, the C-terminal amino acid of a compound of the invention may be the amidated derivative, which is indicated as "-NH₂". Where nothing else is

stated the N-terminal amino acid of a polypeptide comprise a free amino-group, this may also be specified as "H-".

- Where nothing else is specified amino acid can be selected from any amino acid,
5 whether naturally occurring or not, such as alfa amino acids, beta amino acids,
and/or gamma amino acids. Accordingly, the group comprises but are not limited to:
Ala, Val, Leu, Ile, Pro, Phe, Trp, Met, Gly, Ser, Thr, Cys, Tyr, Asn, Gln, Asp, Glu,
Lys, Arg, His Aib, Nal, Sar, Orn, Lysine analogues, DAP, DAPA and 4Hyp.
- 10 Also, according to the invention modifications of the compounds/peptides may be performed, such as glycosylation and/or acetylation of the amino acids.
- Basic amino acid residues are according to invention represented by the residues of
amino acids Arg, Lys, and His, acidic amino acid residues -- by the residues of
15 amino acids Glu and Asp, and hydrophobic amino acid residues by the residues of
amino acids Leu, Ile, Val, Phe, Trp, and Tyr.
- The compound of the invention may comprise two peptide fragments comprising
together between 6 -160 amino acid residues, such as 6-150 amino acid residues,
20 for example 6-140 amino acid residues, such as 6-130 amino acid residues, for
example 6-120 amino acid residues, such as 6-110 amino acid residues, for
example 6-100 amino acid residues, such as 6-90 amino acid residues, for example 6-80
amino acid residues, such as 6-70 amino acid residues, for example 6-60
amino acid residues, such as 6-50 amino acid residues, for example 6-40 amino
25 acid residues, such as 6-30 amino acid residues, for example 6-20 amino acid
residues, such as 6-10 amino acid residues .Thus, according to the invention the
length of amino acid sequences any of the two peptide fragments of the compound
in one embodiment may vary. In another embodiment the length of amino acid
sequence of any of the two peptide fragments of a compound may be the same.
- 30 Accordingly, either of the amino acid sequences may independently consist of 3-80
amino acid residues, such as 3-70, for example 3-60, such as 3-50 amino acid
residues, for example 3-30 amino acid residues, such as 3-20 amino acid residues,
for example 3-15 amino acid residues, such as 3-10 amino acid residues

In another embodiment any of the sequences of the present compound may independently have a length of between 4-80 amino acid residues, such as 4-70, for example 4-60, such as 4-50 amino acid residues, for example 4-40 amino acid residues, such as 4-30 amino acid residues, for example 4-20 amino acid residues, 5 such as 4-15 amino acid residues.

In a further embodiment the sequences may independently have a length of between 5-80 amino acid residues, such as 5-70, for example 5-60, such as 5-50 amino acid residues, for example 5-40 amino acid residues, such as 5-30 amino 10 acid residues, for example 5-20 amino acid residues, such as 5-15 amino acid residues, for example 5-10 amino acid residues.

In yet a further embodiment the sequences may independently have a length of between 6-80 amino acid residues, such as 6-70, for example 6-60, such as 6-50 15 amino acid residues, for example 6-40 amino acid residues, such as 6-30 amino acid residues, for example 6-20 amino acid residues, such as 6-15 amino acid residues, for example 6-10 amino acid residues.

The invention also discloses a compound comprising amino acid sequences, which 20 may independently have a length of between 7-80 amino acid residues, such as 7-70, for example 7-60, such as 7-50 amino acid residues, for example 7-40 amino acid residues, such as 7-30 amino acid residues, for example 7-20 amino acid residues, such as 7-15 amino acid residues, for example 8, 9, 10, 11, 12, 13, or 14 amino acid residues.

25 The length of the sequences may also be of 8-80 amino acid residues, such as 8-70, for example 8-60, such as 8-50 amino acid residues, for example 8-40 amino acid residues, such as 8-30 amino acid residues, for example 8-20 amino acid residues. Or it may be of 9-80 amino acid residues, such as 9-70 amino acid 30 residues, for example 9-60 amino acid residues, such as 9-50 amino acid residues, for example 9-40 amino acid residues, such as 9-30 amino acid residues, for example 9-20 amino acid residues.

A compound which comprises two amino acid sequences, either of which of a length 35 between 10-80 amino acid residues, such as 10-70 amino acid residues, for

example 10-60 amino acid residues, such as 10-50 amino acid residues, for example 10-40 amino acid residues, such as 10-30 amino acid residues, for example 10-20 amino acid residues is also within the scope of the invention.

- 5 In one preferred embodiment a compound comprises two amino acid sequences, either of which or both may independently have a length of between 15-80 amino acid residues, such as 15-70 amino acid residues, for example 15-60 amino acid residues, such as 15-50 amino acid residues, for example 15-40 amino acid residues, such as 15-30 amino acid residues, for example 15-20.

- 10 In another preferred embodiment of the invention the minimal length of any of the two of amino acid sequences of the compound may independently be of 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acid residues, and the maximal is 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acid residues.

- 15 In still another preferred embodiment a length of a peptide fragment of the compound may independently be of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues.

- 20 In yet another preferred embodiment of the invention at least one of the two peptide sequences of the compound may derive from the sequence of a polypeptide selected from the group comprising cell adhesion molecules, cell-surface receptors, heparan sulphate proteoglycans, and metalloproteases, extracellular matrix molecules or growth factors.

- 25 According to the invention the cell adhesion molecule may be selected from the group comprising

- Neural Cell Adhesion Molecule (NCAM) (Swiss-Prot Ass. Nos: P13591, P13595-01, P13595),

- 30 - Neural cell adhesion molecule L1 (Swiss-Prot Ass. Nos: Q9QYQ7, Q9QY38, P11627, Q05695, P32004),

- Neural Cell Adhesion Molecule-2 (NCAM-2) (Swiss-Prot Ass. No: P36335)

- Neuron-glia Cell Adhesion Molecule (Ng-CAM) (Swiss-Prot Ass. No: Q03696; Q90933),

- 35 - Neural cell adhesion molecule CALL (Swiss-Prot Ass. No: O00533),

- Neuroglan (Swiss-Prot Ass. No: P91767, P20241),
 - Nr-CAM (HBRAVO, NRCAM, NR-CAM 12) (Swiss-Prot Ass. Nos: Q92823, O15179, Q9QVN3)
 - Axonin-1/TAG-1 (Swiss-Prot Ass. Nos: Q02246, P22063, P28685),
 - 5 - Axonal-associated Cell Adhesion Molecule (AxCAM) (NCBI Ass. No: NP_031544.1; Swiss-Prot Ass. No: Q8TC35),
 - Myelin-Associated Glycoprotein (MAG) (Swiss-Prot Ass. No: P20917),
 - Neural cell adhesion molecule BIG-1 (Swiss-Prot Ass. No: Q62682),
 - Neural cell adhesion molecule BIG-2 (Swiss-Prot Ass. No: Q62845),
 - 10 - Fasciclin (FAS-2) (Swiss-Prot Ass. No: P22648),
 - Neural cell adhesion molecule HNB-3/NB-3 (Swiss-Prot Ass. Nos: Q9UQ52, P97528, Q9JMB8)
 - Neural cell adhesion molecule HNB-2/NB-2 (Swiss-Prot Ass. Nos: O94779, P07409, P97527),
 - 15 - Cadherin (Swiss-Prot Ass. No: Q9VW71),
 - Junctional Adhesion Molecule-1 (JAM-1) (Swiss-Prot Ass. Nos: Q9JKD5, O88792),
 - Neural cell adhesion F3/F11(Contactin) (Swiss-Prot Ass. Nos: Q63198, P1260, Q12860, Q28106, P14781, O93250),
 - 20 - Neurofascin (Swiss-Prot Ass. Nos: Q90924, Q91Z60; O42414),
 - B-lymphocyte cell adhesion molecule CD22 (Swiss-Prot Ass. Nos: Q9R094, P20273),
 - Neogenin (NEO1) (Swiss-Prot Ass. Nos: Q92859, P97603, Q90610, P97798),
 - Intercellular Cell Adhesion Molecule-5 (ICAM-5/telencephalin) (Swiss-Prot Ass. Nos: Q8TAM9, Q60625) or
 - 25 - Galactose binding lectin-12 (galectin-12) (Swiss-Prot Ass. Nos: Q91VD1, Q9JKX2, Q9NZ03),
 - Galactose binding lectin-4 (galectin-4) (Swiss-Prot Ass. No: Q8K419; P38552), or fragments, or variants thereof.
- 30 The functional cell-surface receptor may selected from the group comprising
- Fibroblast Growth Factor Receptor 1 (FGFR1) (Swiss-Prot Ass. Nos: Q9QZM7, Q99AVV7, Q9UD50, Q63827),
 - Fibroblast Growth Factor Receptor 2 (FGFR2) (Swiss-Prot Ass. Nos: Q96KM2, P21802, Q63241),
- 35

- Fibroblast Growth Factor Receptor 3 (FGFR3) (Swiss-Prot Ass. Nos: Q95M13, AF487554, Q99052),
- Fibroblast Growth Factor Receptor 4 (FGFR4) (Swiss-Prot Ass. No: Q91742),
- Neurotrophin Tyrosin Kinase Type-2 (NTRKT-2) (Swiss-Prot Ass. No: Q8WXJ5),
- Leukocyte Antigen Related Protein-Tyrosine Phosphatase (LAR-PTPRF) (Swiss-Prot Ass. Nos: Q9EQ17, Q64605, Q64604, Q9QW67, Q9VIS8 P10586),
- Nephrin (Swiss-Prot Ass. Nos: Q925S5, Q9JIX2, Q9ET59, Q9R044, Q9QZS7, Q06500),
- Protein-Tyrosine Phosphatase Receptor type S (PTPRS) (Swiss-Prot Ass. Nos: Q64699, Q13332, Q75870),
- Protein-Tyrosine Phosphatase Receptor type kappa (R-PTP-kappa) (Swiss-Prot Ass. No: Q15262),
- Protein-Tyrosine Phosphatase Receptor type D (PTPRD) (Swiss-Prot Ass. Nos: Q8WX65, Q9IAJ1, P23468, Q64487),
- Ephrin type-A receptor 8 (EPHA8/Tyrosine-Protein Kinase Receptor EEK) (Swiss-Prot Ass. Nos: O09127, P29322),
- Ephrin type-A receptor 3 (EPHA8/Tyrosine-Protein Kinase Receptor ETK-1/CEK4) (Swiss-Prot Ass. No: P29318),
- Ephrin type-A receptor 2 (Swiss-Prot Ass. No: Q8N3Z2)
- Insulin Receptor (IR) (Swiss-Prot Ass. No: Q9PWN6)
- Insulin-like Growth Factor-1 Receptor (IGF-1) (Swiss-Prot Ass. Nos: Q9QVW4, P08069, P24062, Q60751, P15127, P15208)
- Insulin-related Receptor (IRR) (Swiss-Prot Ass. No: P14616),
- Tyrosine-Protein Kinase Receptor Tie-1 (Swiss-Prot Ass. Nos: 06805, P35590, Q06806),
- Roundabout receptor-1 (robo-1) (Swiss-Prot Ass. Nos: O44924, AF041082, Q9Y6N7),
- Neuronal nicotinic acetylcholine receptor alpha 3 subunit (CHRNA3) (Swiss-Prot Ass. Nos: Q8VHH6; P04757, Q8R4G9, P32297)
- Neuronal acetylcholine receptor alpha 6 subunit (Swiss-Prot Ass. Nos: Q15825, Q9R0W9)
- Platelet-Derived Growth Factor Receptor Beta (PDGFRB) (Swiss-Prot Ass. Nos: Q8R406, Q05030),

- Interleukin-6 Receptor (IL-6R) (Swiss-Prot Ass. No: Q00560),
 - Interleukin-23 Receptor (IL-23R) (Swiss-Prot Ass. No: AF461422),
 - Beta-common cytokine receptor of IL-3, IL5 and GmCsf (Swiss-Prot Ass. No: P32927)
- 5 - Cytokine Receptor-Like molecule 3 (CRLF1) (Swiss-Prot Ass. No: Q9JM58),
- Class I Cytokine Receptor (ZCYTOR5) (Swiss-Prot Ass. No: Q9UHH5)
 - Netrin-1 receptor DCC (Swiss-Prot Ass. No: P43146),
 - Leukocyte Fc Receptor-like Protein (IFGP2) (Swiss-Prot Ass. Nos: Q96PJ6, Q96KM2),
- 10 - Macrophage Scavenger Receptor 2 (MSR2) (Swiss-Prot Ass. No: Q91YK7), or
- Granulocyte Colony Stimulating Factor Receptor (G-CSF-R) (Swiss-Prot Ass. No: Q99062),
or fragments, or variants thereof.
- 15 The heparan sulphate proteoglycan according to the invention is perlecan (Swiss-Prot Ass. No: P98160), or a fragment, or a variant thereof.
- The metalloprotease may be selected from the group comprising
- ADAM-8 (Swiss-Prot Ass. No: Q05910),
- 20 - ADAM-19 (Swiss-Prot Ass. Nos: Q9H013, O35674),
- ADAM-8 (Swiss-Prot Ass. No: P78325),
 - ADAM-12 (Swiss-Prot Ass. Nos: O43184, Q61824),
 - ADAM-28 (Swiss-Prot Ass. Nos: Q9JLN6, Q61824, Q9XSL6, Q9UKQ2),
 - ADAM-33 precursor (Swiss-Prot Ass. Nos: Q8R533, Q923W9),
- 25 - ADAM-9 (Swiss-Prot Ass. Nos: Q13433, Q61072),
- ADAM-7 (Swiss-Prot Ass. NoS: Q9H2U9, O35227, Q63180),
 - ADAM-1A Fertilin alpha (Swiss-Prot Ass. No: Q8R533),
 - ADAM-15 (Swiss-Prot Ass. Nos: Q9QYV0, O88839, Q13444),
- 30 - Metalloproteinase-desintegrin domain containing protein (TECAM) (Swiss-Prot Ass. No: AF163291),
- Metalloproteinase 1 (Swiss-Prot Ass. Nos: O95204, Q9BSI6),
- or fragments, or variants thereof.
- The extracellular matrix molecule may selected from the group comprising
- 35 - Collagen type VII (Swiss-Prot Ass. No: Q63870),

- Fibronectin (Swiss-Prot Ass. Nos: Q95KV4, Q95KV5, P07589, Q28377, U42594, O95609, P11276), or
- Tenascin-R (Swiss-Prot Ass. Nos: Q15568, O00531, Q90995, P10039), or fragments, or variants thereof.

5

The growth factor according to the invention is Cytokine-like factor-1 (CLF-1) (Swiss-Prot Ass. No:O75462), or a fragment, or a variant thereof.

10 The term "fragment" in the present context is meant a polypeptide having the amino acid sequence equal to 1 - 99 % of the length of the predetermined protein/polypeptide amino acid sequence, said polypeptide being a functional homologue of the predetermined polypeptide selected from the above groups.

15 The term "variant" in the present content is meant a polypeptide having the amino acid sequence, which i) is 25-100 % homologous to the sequence of the predetermined polypeptide, and/or ii) comprises other chemical moieties, such various post-translational modifications of amino acid residues, for example phosphorylation, acylation, glycosilation of the amino acids residues, and/or iii) is associated with another biological molecule(s), for example a polypeptide(s), lipid(s) or carbohydrate(s), said polypeptide being a functional homologue of the predetermined polypeptide selected from the above groups

20 By the term "functional homologue" of a predetermined polypeptide is in the present context meant a molecule which is capable of one or more biological functions of the predetermined polypeptide, in preferred embodiment is capable of binding to a cell surface receptor and thereby modulating said receptor dependent signal transduction.

25 A preferred cell surface receptor of the invention is a receptor selected from the family of fibroblast growth factor receptors (FGFRs), and the molecules defined above all are the members of a new group of FGFR ligands. The above molecules according to the invention comprise a binding site for FGFR, said site having structural characteristics of the FGFR binding site of NCAM.

In particular, according to the invention the FGFR binding site of the above molecules comprises an amino acid sequence that has at least 60 %, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95% homology to the sequence EVYVVAENQQGKSKA (SEQ ID NO 1), the FGL peptide of NCAM. The homology of one amino acid sequence with another amino acid is defined as a percentage of identical amino acids in the two collated sequences.

5 In another embodiment, the FGFR binding site of the above molecules comprises an
10 amino acid sequence that has an amino acid sequence having at least 60 %, more
preferably at least 70%, more preferably at least 80%, more preferably at least 90%,
more preferably 95% positive amino acid matches compared to to the sequence
EVYVVAENQQGKSKA (SEQ ID NO 1). A positive amino acid match is defined as
an identity or similarity defined by physical and/or chemical properties of the amino
15 acids having the same position in two compared sequences. Preferred positive
amino acid matches of the present invention are K to R, E to D, L to M, Q to E, I to
V, I to L, A to S, Y to W, K to Q, S to T, N to S and Q to R.

20 In still another embodiment the FGFR binding site of the above molecules is ac-
cording to invention may be characterised by specific 3D-structural features. The
invention preferably features a binding site, which essentially consists of one or
more "strand-loop-strand" structural motifs.

25 In one preferred embodiment at least one of the at least two amino acid sequences
comprising the structural motif defined above and/or derived from the above mole-
cules may be selected from any of the following peptide sequences:

EVYVVAENQQGKSKA (SEQ ID NO 1),
NIEVVVEAENALGKKV (SEQ ID NO: 2),
ATNRQGKVKAFAHL (SEQ ID NO: 3),
30 RYVELYVVADSQEFAQK (SEQ ID NO: 4)
VAENSRGKNVAKG (SEQ ID NO: 5),
GEYWCVVAENQYGQR (SEQ ID NO: 6),
RLAALNGKGLGEIS (SEQ ID NO: 7),
KYIAENMKAQNVAKEI (SEQ ID NO: 8),
35 TIMGLKPETRYAVR (SEQ ID NO: 9),

KGLGEISAATEFKT (SEQ ID NO: 10),
NMGIWVQAENALG (SEQ ID NO: 11),
IWVQAENMLG (SEQ ID NO: 12),
EIWVEATNRLG (SEQ ID NO: 13),
5 VWVQAANALG (SEQ ID NO: 14),
EVWIEKDPAKGRI (SEQ ID NO: 15),
ATNKGGEVKKNGHL (SEQ ID NO: 16),
KYVELYLVADYLEFQK (SEQ ID NO: 17),
RYVELYVVVDNAEFQ (SEQ ID NO: 18),
10 KYVELVIVADNREFQR (SEQ ID NO: 19),
KYIEYYLVLDNGEFKR (SEQ ID NO: 20),
RYLELYIVADHTLF (SEQ ID NO: 21),
KYVEMFVVVNHQRFQ (SEQ ID NO: 22),
RYVELFIIVVDKERY (SEQ ID NO: 23),
15 KYVELFIVADDTVYRR (SEQ ID NO: 24),
KFIELFVVADEYYVYRR (SEQ ID NO: 25),
KIVEKVIVADNSEVRK (SEQ ID NO: 26),
VELVIVADHSEAQK (SEQ ID NO: 27),
VAENSRGKNIAKG (SEQ ID NO: 28),
20 IAENSRGKNVARG (SEQ ID NO: 29),
AENSRGKNSFRG (SEQ ID NO: 30),
IASNLRGRNLAKG (SEQ ID NO: 31),
IPENSLGKTYAKG (SEQ ID NO: 32),
IAENMKAQNEAK (SEQ ID NO: 33),
25 QFIAENMKSHNETKEV (SEQ ID NO: 34),
GEYWCVAKNRVGQ (SEQ ID NO: 35),
GSYTCVAENMVGK (SEQ ID NO: 36),
GKYVCVGTNMVGER (SEQ ID NO: 37),
GNYTCVVENEYG (SEQ ID NO: 38),
30 GEYTCLAGNSIG (SEQ ID NO: 39),
QYYCVAENGYG (SEQ ID NO: 40),
GEYYQEAEQNGYG (SEQ ID NO: 41),
GNYTCLVENEYG (SEQ ID NO: 42),
GMYQCLAENAYG (SEQ ID NO: 43),
35 GMYQCAENTHG (SEQ ID NO: 44),

GIYYCLASNNYG (SEQ ID NO: 45),
GGYYCTADNSYG (SEQ ID NO: 46),
GEYQCFARNDYG (SEQ ID NO: 47),
GEYFCLASNKMG (SEQ ID NO: 48),
5 GEYQCFARNKFG (SEQ ID NO: 49),
GEYFCLASNKMG (SEQ ID NO: 50),
GGYYCTADNNYG (SEQ ID NO: 51),
GNYSCAEENAWGTK (SEQ ID NO: 52),
GEYTCLAENSLG (SEQ ID NO: 53),
10 GEYECAENGRLG (SEQ ID NO: 54),
GNYTCVVENKFGR (SEQ ID NO: 55),
GEYTCLAGNSIG (SEQ ID NO: 56),
GEYFCVASNPIG (SEQ ID NO: 57),
EYTCIANNQAGE (SEQ ID NO: 58),
15 GMYQCVAENKHLG (SEQ ID NO: 59),
GEYMCTASNTIGQ (SEQ ID NO: 60),
EYVCIAENKAGEQ (SEQ ID NO: 61),
GDYTLIAKNEYGK (SEQ ID NO: 62),
GFYQCVAENEAG (SEQ ID NO: 63),
20 GKYECAATNSAGTR (SEQ ID NO: 64),
GEYFCVYNNSLG (SEQ ID NO: 65),
GEYECAATNAHGR (SEQ ID NO: 66),
GAYWCQGTNSVGK (SEQ ID NO: 67),
GTYSCVAENILG (SEQ ID NO: 68),
25 RVAAVNGKGQGDYS (SEQ ID NO: 69),
RVAAINCGIGPFS (SEQ ID NO: 70),
AVLNGKGLG (SEQ ID NO: 71),
ALNGQGLGATS (SEQ ID NO: 72),
RLAAKNRAGLGE (SEQ ID NO: 73),
30 RLGVTGKDLGEI (SEQ ID NO: 74),
TVTGLKPSTSYMVK (SEQ ID NO: 75),
TLTGLKPSTRYRI (SEQ ID NO: 76),
TLTGLQPSTRYRV (SEQ ID NO: 77),
TLLGLKPDPTYDIK (SEQ ID NO: 78),
35 TLQGLRPETAYELR (SEQ ID NO: 79),

TLRGLRPETAYELR (SEQ ID NO: 80),
TLMNLRPKTGYSVR (SEQ ID NO: 81),
TVSGLKPGTRY (SEQ ID NO: 82),
TISGLKPDPTY (SEQ ID NO: 83),
5 TLQGLKPDTAY (SEQ ID NO: 84),
LRGLKPWTQYAV (SEQ ID NO: 85),
IDGLEPDTEYIVR (SEQ ID NO: 86),
LQGLKPWTQYAI (SEQ ID NO: 87),
TITGLEPGTEYTIQ (SEQ ID NO: 88),
10 GLKPWTQYAV (SEQ ID NO: 89),
TLASLKPWTQYAV (SEQ ID NO: 90),
LMGLQPATEYIV (SEQ ID NO: 91),
KGGMGPMSEAVQFRT (SEQ ID NO: 92),
TLTGLKPDTTYDVK (SEQ ID NO: 93),
15 ISGLQPETSYSI (SEQ ID NO: 94),
TLLGLKPDTTYDIK (SEQ ID NO: 95),
TISGLTPETTYSI (SEQ ID NO: 96),
GNYSCLAENRLGR (SEQ ID NO: 97),
GNYTCVVENRVG (SEQ ID NO: 98),
20 GTYHCVATNAHG (SEQ ID NO: 99),
LSHNGVLTGYLLSY (SEQ ID NO: 100),
NGVLTGYVLRY (SEQ ID NO: 101),
NGVLTGYNLRY (SEQ ID NO: 102),
NGNLTGYLLQY (SEQ ID NO: 103),
25 VDENGVLTGYKIYY (SEQ ID NO: 104),
THNGALVGYSVRY (SEQ ID NO: 105),
NGILTEYILKY (SEQ ID NO: 106),
NGILIGYTLRY (SEQ ID NO: 107),
THSGQITGYKIRY (SEQ ID NO: 108),
30 NGKITGYIIYY (SEQ ID NO: 109),
LSHNGIFTLY (SEQ ID NO: 110),
NGILTEYTLKY (SEQ ID NO: 111),
LDPNGIITQYEISY (SEQ ID NO: 112),
NGKITGYIIYY (SEQ ID NO: 113),
35 HLEVQAFNGRGSGPA (SEQ ID NO: 114),

HLTVRAYNGAGYGP (SEQ ID NO: 115),
HLSVKAYNSAGTGPS (SEQ ID NO: 116),
HLAVKAYNSAGTGPS (SEQ ID NO: 117),
NLEVRAFNSAGDGP (SEQ ID NO: 118),
5 HLTVALAYNSKGAGP (SEQ ID NO: 119),
LRVLVFNGRGDGP (SEQ ID NO: 120),
HIDVSAFNSAGYGP (SEQ ID NO: 121),
HAAELFNKR (SEQ ID NO: 122),
LELQSINFLGGQPA (SEQ ID NO: 123),
10 HFTVRAYNGAGYGP (SEQ ID NO: 124),
HLEVQAFNGRGSQPA (SEQ ID NO: 125),
VIADQPTFKYLIK (SEQ ID NO: 126),
TIKGLRPGVVYEGQ (SEQ ID NO: 127),
TLTELPSTQYTVK (SEQ ID NO: 128),
15 TLDDLAPDTTYLVQ (SEQ ID NO: 129),
TVSDVTPHAIYTVR (SEQ ID NO: 130),
IIRGLNASTRYLFR (SEQ ID NO: 131),
TLMNLRPKTGYSVR (SEQ ID NO: 132),
TLTGLKPGTEYEVR (SEQ ID NO: 133),
20 GPEHLMPSSTYVAR (SEQ ID NO: 134),
RVTLTPKKTYEFR (SEQ ID NO: 135),
LTGLKPGTEYEFR (SEQ ID NO: 136),
EVRVQAVNGGGNGPP (SEQ ID NO: 137),
LIKVAINDRGE (SEQ ID NO: 138),
25 VVSIIAVNGREE (SEQ ID NO: 139),
VVSVYAQQNQNGE (SEQ ID NO: 140),
TISLVAEKGRHK (SEQ ID NO: 141),
HLEVQAFNGRGSQPA (SEQ ID NO: 142),
HVEVQAFNGRGLGPA (SEQ ID NO: 143),
30 HVEVQAFNGRGLGPA (SEQ ID NO: 144),
EFRVRAVNGAGEG (SEQ ID NO: 145), or
VARVRTRLAPGSRLS (SEQ ID NO: 146), or
or fragments, or variants, or homologues thereof.

In another preferred embodiment at least one of the at least two peptide sequences may be selected from the group consisting of

EVYVVAENQQGKSKA (SEQ ID NO 1),
NIEVWVEAENALGKKV (SEQ ID NO: 2),
5 ATNRQGKVKAFAHL (SEQ ID NO: 3),
RYVELYVVADSQEFQK (SEQ ID NO: 4)
VAENSRGKNVAKG (SEQ ID NO: 5),
GEYWCVAENQYGQR (SEQ ID NO: 6),
RLAALNGKGLGEIS (SEQ ID NO: 7),
10 KYIAENMKAQNVAKEI (SEQ ID NO: 8),
TIMGLKPETRYAVR (SEQ ID NO: 9),
KGLGEISAATEFKT (SEQ ID NO: 10),
or fragments, or variants, or homologues thereof.

15 In still another preferred embodiment at least one of the at least two peptide sequences may be selected from the group consisting of

NMGIWVQAENALG (SEQ ID NO: 11),
IWVQAENMLG (SEQ ID NO: 12),
20 EIWVEATNRLG (SEQ ID NO: 13),
VVVQAANALG (SEQ ID NO: 14),
EWVIEKDPAKGRI (SEQ ID NO: 15),
ATNKGGEVKKNGHL (SEQ ID NO: 16),
KYVELYLVADYLEFQK (SEQ ID NO: 17),
25 RYVELYVVVDNAEFQ (SEQ ID NO: 18),
KYVELVIVADNREFQR (SEQ ID NO: 19),
KYIEYYLVLDNGEFKR (SEQ ID NO: 20),
RYLELYIVADHTLF (SEQ ID NO: 21),
KYVEMFVVVNHQRFQ (SEQ ID NO: 22),
30 RYVELFIVVDKERY (SEQ ID NO: 23),
KYVELFIVADDTVYRR (SEQ ID NO: 24),
KFIELFVVADEYYVYRR (SEQ ID NO: 25),
KIVEKVIVADNSEVRK (SEQ ID NO: 26),
VELVIVADHSEAQK (SEQ ID NO: 27),
35 VAENSRGKNIAKG (SEQ ID NO: 28),

IAENSRGKNVARG (SEQ ID NO: 29),
AENSRGKNSFRG (SEQ ID NO: 30),
IASNLRGRNLAKG (SEQ ID NO: 31),
IPENSLGKTYAKG (SEQ ID NO: 32),
5 IAENMKAQNEAK (SEQ ID NO: 33),
QFIAENMKSHNETKEV (SEQ ID NO: 34),
GEYWCVAKNRVGQ (SEQ ID NO: 35),
GSYTCVAENMVGK (SEQ ID NO: 36),
GKYVCVGTNMVGER (SEQ ID NO: 37),
10 GNYTCVVENEYG (SEQ ID NO: 38),
GEYTCLAGNSIG (SEQ ID NO: 39);
QYYCVAENGYG (SEQ ID NO: 40),
GEYYQEAEQNGYG (SEQ ID NO: 41),
GNYTCLVENEYG (SEQ ID NO: 42),
15 GMYQCLAENAYG (SEQ ID NO: 43),
GMYQCAENTHG (SEQ ID NO: 44),
GIYYCLASNNYG (SEQ ID NO: 45),
GGYYCTADNSYG (SEQ ID NO: 46),
GEYQCFARNDYG (SEQ ID NO: 47),
20 GEYFCLASNMG (SEQ ID NO: 48),
GEYQCFARNKFG (SEQ ID NO: 49),
GEYFCLASNMG (SEQ ID NO: 50),
GGYYCTADNNYG (SEQ ID NO: 51),
GNYSCEAENAWGTK (SEQ ID NO: 52),
25 GEYTCLAENSLG (SEQ ID NO: 53),
GEYECVAENGRLG (SEQ ID NO: 54),
GNYTCVVENKFGR (SEQ ID NO: 55),
GEYTCLAGNSIG (SEQ ID NO: 56),
GEYFCVASNPIG (SEQ ID NO: 57),
30 EYTCIANNQAGE (SEQ ID NO: 58),
GMYQCVAENKHLG (SEQ ID NO: 59),
GEYMCTASNTIGQ (SEQ ID NO: 60),
EYVCIAENKAGEQ (SEQ ID NO: 61),
GDYTLIAKNEYGK (SEQ ID NO: 62),
35 GFYQCVAENEAG (SEQ ID NO: 63),

GKYECVATNSAGTR (SEQ ID NO: 64),
GEYFCVYNNSLG (SEQ ID NO: 65),
GEYECAATNAHGR (SEQ ID NO: 66),
GAYWCQGTNSVGK (SEQ ID NO: 67),
5 GTYSCVAENILG (SEQ ID NO: 68),
RVAAVNGKGQGDYS (SEQ ID NO: 69),
RVAAINCGIGPFS (SEQ ID NO: 70),
AVLNGKGLG (SEQ ID NO: 71),
ALNGQGLGATS (SEQ ID NO: 72),
10 RLAAKNRAGLGE (SEQ ID NO: 73),
RLGVVTGKDLGEI (SEQ ID NO: 74),
TVTGLKPETSYMVK (SEQ ID NO: 75),
TLTGLKPSTRYRI (SEQ ID NO: 76),
TLTGLQPSTRYRV (SEQ ID NO: 77),
15 TLLGLKPDTTYDIK (SEQ ID NO: 78),
TLQGLRPETAYELR (SEQ ID NO: 79),
TLRGLRPETAYELR (SEQ ID NO: 80),
TLMNLRPKTGYSVR (SEQ ID NO: 81),
TVSGLKPGTRY (SEQ ID NO: 82),
20 TISGLKPDTTY (SEQ ID NO: 83),
TLQGLKPDTAY (SEQ ID NO: 84),
LRGLKPWTQYAV (SEQ ID NO: 85),
IDGLEPDTEYIVR (SEQ ID NO: 86),
LQGLKPWTQYAI (SEQ ID NO: 87),
25 TITGLEPGTEYTIQ (SEQ ID NO: 88),
GLKPWTQYAV (SEQ ID NO: 89),
TLASLKPWTQYAV (SEQ ID NO: 90),
LMGLQPATEYIV (SEQ ID NO: 91),
KGMGPMSEAVQFRT (SEQ ID NO: 92),
30 TLTGLKPDTTYDVK (SEQ ID NO: 93),
ISGLQPETSYSL (SEQ ID NO: 94),
TLLGLKPDTTYDIK (SEQ ID NO: 95),
TISGLTPETTYSI (SEQ ID NO: 96),
GNYSCLAENRLGR (SEQ ID NO: 97),
35 GNYTCVVENRVG (SEQ ID NO: 98),

GTYHCVATNAHG (SEQ ID NO: 99),
LSHNGVLTGYLLSY (SEQ ID NO: 100),
NGVLTGVLRY (SEQ ID NO: 101),
NGVLTGYNLRY (SEQ ID NO: 102),
5 NGNLTGULLQY (SEQ ID NO: 103),
VDENGVLTGYKIYY (SEQ ID NO: 104),
THNGALVGYSVRY (SEQ ID NO: 105),
NGILTEYILKY (SEQ ID NO: 106),
NGILIGYTLRY (SEQ ID NO: 107),
10 THSGQITGYKIRY (SEQ ID NO: 108),
NGKITGYIYYY (SEQ ID NO: 109),
LSHNGIFTLY (SEQ ID NO: 110),
NGILTEYTLKY (SEQ ID NO: 111),
LDPNGIITQYEISY (SEQ ID NO: 112),
15 NGKITGYIYYY (SEQ ID NO: 113),
HLEVQAFNGRGS GPA (SEQ ID NO: 114),
HTVRAYNGAGYGP (SEQ ID NO: 115),
HLSVKAYNSAGTGPS (SEQ ID NO: 116),
HLAVKAYNSAGTGPS (SEQ ID NO: 117),
20 NLEVRAFNSAGDGP (SEQ ID NO: 118),
HTVLAYNSKGAGP (SEQ ID NO: 119),
LRVLVFNGRGDGP (SEQ ID NO: 120),
HIDVSAFNSAGYGP (SEQ ID NO: 121),
HIAVELFNGR (SEQ ID NO: 122),
25 LELQSINFLGGQPA (SEQ ID NO: 123),
HTVRAYNGAGYGP (SEQ ID NO: 124),
HLEVQAFNGRGSQPA (SEQ ID NO: 125),
VIADQPTFVKYLIK (SEQ ID NO: 126),
TIKGLRPGVVYEGQ (SEQ ID NO: 127),
30 TLTELSPSTQYTVK (SEQ ID NO: 128),
TLDDLAPDTTYLVQ (SEQ ID NO: 129),
TVSDVTPHAIYTVR (SEQ ID NO: 130),
IIRGLNASTRYLFR (SEQ ID NO: 131),
TLMNLRPKTGYSVR (SEQ ID NO: 132),
35 TLTGLKPGTEYEVR (SEQ ID NO: 133),

- GPEHLMPSSTYVAR (SEQ ID NO: 134),
RVTGLTPKKTYEFR (SEQ ID NO: 135),
LTGLKPGTEYEFR (SEQ ID NO: 136),
EVRVQAVNGGGNGPP (SEQ ID NO: 137),
5 LIKVVAINDRGE (SEQ ID NO: 138),
VVSIIAVNGREE (SEQ ID NO: 139),
VVSVYAQQNQNGE (SEQ ID NO: 140),
TISLVAEKGRHK (SEQ ID NO: 141),
HLEVQAFNngrgsgpa (SEQ ID NO: 142),
10 HVEVQAFNngrglgpa (SEQ ID NO: 143),
HVEVQAFNngrglgpa (SEQ ID NO: 144),
EFRVRAVNGAGEG (SEQ ID NO: 145), or
VARVRTRLAPGSRLS (SEQ ID NO: 146), or
or fragments, or variants, or homologues thereof.
15
The present invention relates to fragments of the above peptide sequences having at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 95% of the length of a predetermined sequence set forth in
20 SEQ ID NOS: 1-146, wherein an amino acid sequence homology between a fragment and the predetermined sequence is 100%. A variant in the present context is defined as an amino acid sequence having at least 60 %, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95% homology to a sequence selected from SEQ ID NOS: 1-146, or an amino acid
25 sequence having at least 60 %, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95% positive amino acid matches compared to a sequence selected from SEQ ID NOS: 1-146. A positive amino acid match is defined as an identity or similarity defined by physical and/or chemical properties of the amino acids having the same position in two compared
30 sequences. Preferred positive amino acid matches of the present invention are K to R, E to D, L to M, Q to E, I to V, I to L, A to S, Y to W, K to Q, S to T, N to S and Q to R. The homology of one amino acid sequence with another amino acid is defined as a percentage of identical amino acids in the two collated sequences. A homologue in the present context is defined as an amino acid sequence which has less than
35 60% and more than 19 %, such as 50-59%, for example 55%, such as 40-49%, for

- example 45%, such as 30-39%, for example 35%, such as 20-29%, for example 25% homology to any of the sequences set forth in SEQ ID NOS: 1-146 having remained some of the physical properties of the predetermined sequences, such as for example the three-dimensional structure or some of the functional properties,
- 5 such as for example a capability to interact with another molecule, in particular with a receptor molecule. A variant of a homologue in the present context is defined as an amino acid sequence having at least 60 %, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably 95% positive amino acid matches compared to a homologue of any of the sequences selected
- 10 from SEQ ID NOS: 1-146. Preferred embodiments of the positive amino acid matches is as the defined above. The invention concerns the fragments, variants and homologues, which remain a capability of the predetermined sequences to interact with the cell-surface receptor defined below.
- 15 It is preferred, if the selected peptide sequence is EVYVVAENQQGKSKA (SEQ ID NO: 1). In another preferred embodiment the sequence is NIEWWVEAENALGKKV (SEQ ID NO: 2).
- According to the invention, the compound may comprise at least two different peptide sequences selected from any of the above peptide sequences (SEQ ID NO: 1-20 146). Yet, the compound may comprise at least two the above peptide sequences having the identical amino acid sequence.
- In one preferred embodiment the compound comprises at least two identical peptide sequences having the sequence EVYVVAENQQGKSKA (SEQ ID NO: 1). In another preferred embodiment the compound comprises at least two identical peptide sequences having the sequence is NIEWWVEAENALGKKV (SEQ ID NO: 2). In still 25 another preferred embodiment the compound comprises at least two different peptide sequences one of which is having the sequence EVYVVAENQQGKSKA (SEQ ID NO: 1), and the other is having the sequence NIEWWVEAENALGKKV (SEQ ID NO: 2). Other preferred embodiments encompass compounds comprising at least 30 two amino acid sequences comprising fragments, variants or homologues of SEQ ID NO: 1 or 2, wherein said fragments, variants and homologues being as defined above.

- In still another preferred embodiment at least one of at least two amino sequences of a compound comprise; essentially comprise, or consist of two or more peptide sequences of SEQ ID NOS: 1-146 co-joined in one peptide chain. In other preferred embodiments, the co-joined peptide sequences may be identical, such as for example SEQ ID NO: 1 repeated in a peptide chain 2 to 5 times, for example SEQ ID NO: 2 repeated in a peptide chain 2 to 5 times, or for example any of the sequences SEQ ID NO:3-146 repeated in a peptide chain 1 5 times such as an oligomer (multimer) of the identical monomers. In other embodiments the peptide fragments forming an oligomer may differ in the amino acid sequence, such as for example the oligomer (dimer) consisting of two monomers, one of which is having the amino acid sequence set forth in SEQ ID NO: 1 and another in SEQ ID NO: 2, or for example any of the sequences set forth in SEQ ID NOS: 3-146. Thus, according to invention, an oligomer (multimer) of may for example consists of 2, 3, 4, 5, 6, 7, 8, 9 or 10 sequences (monomers).
- A compound consisting of two peptide sequences connected to each other through a linker, wherein each of said peptide sequences is consisting a single copy any of the sequences identified in SEQ ID NOS:1-146, is a preferred compound of the invention. Even more preferred compound is a compound as above, wherein two peptide sequences both are having the sequence set forth in SEQ ID NO:1, or a compound, whereing the peptide sequence is SEQ ID NO: 2.
- Orientation of peptide sequences in the compound may be either N to C, C to N or mixed. The term "N to C orientation" is meant that two predetermined sequences are connected to a linker through their N-terminal amino acid residue, such as a compound of the type:(COOH)peptide sequence(NH2)-linker-(NH2)peptide sequence(COOH). The term "C to N orientation" is meant that two predetermined sequences are connected to a linker through their C-terminal amino acid residue, such as a compound of the type:(NH2)peptide sequence(COOH)-linker-(COOH)peptide sequence(NH2). The term "mixed orientation" is meant that two predetermined sequences are connected to a linker either through their C-terminal amino acid residue or N-terminal, such as one peptide sequence though its N-terminal amino acid residue and another through its C-terminal amino acid residue, for example as a compound of the type:(NH2)peptide sequence(COOH)-linker-(NH2)peptide sequence(COOH).

1.2 Linker

According to invention the at least two peptide sequences are co-joined in a compound by a linker. A linker molecule is according to the invention is selected from
5 achiral di-, tri- or tetracarboxylic acids, said acids having the general formula



wherein

- 10 n and m independently are an integer of from 1 to 20,
X is HN, $H_2N(CR_2)pCR$, $RHN(CR_2)pCR$, $HO(CR_2)pCR$, $HS(CR_2)pCR$, halogen-
 $(CR_2)pCR$, $HOOC(CR_2)pCR$, $ROOC(CR_2)pCR$, $HCO(CR_2)pCR$, $RCO(CR_2)pCR$,
[$HOOC(A)n$][$HOOC(B)m$]CR(CR_2)pCR, $H_2N(CR_2)p$, $RHN(CR_2)p$, $HO(CR_2)p$,
 $HS(CR_2)p$, halogen-(CR_2)p, $HOOC(CR_2)p$, $ROOC(CR_2)p$, $HCO(CR_2)p$, $RCO(CR_2)p$,
15 or [$HOOC(A)n$][$HOOC(B)m$](CR_2)p , wherein p is 0 or integer of from 1 to 20,
A and B independently are a substituted or unsubstituted C_{1-10} alkyl, a substituted or
unsubstituted C_{2-10} alkenyl, a substituted or unsubstituted cyclic moiety, a substituted
or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety,
or A and B together form a substituted or unsubstituted cyclic moiety, substituted or
20 unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

Under the term C_{1-10} alkyl is meant straight or branched chain alkyl groups having 1-
10 carbon atoms, e.g. methyl, ethyl, isopropyl, butyl, and tertbutyl.

- 25 Under the term C_{2-10} alkenyl is meant straight or branched chain alkenyl groups
having 2-10 carbon atoms, e.g. ethynyl, propenyl, isopropenyl, butenyl, and tert-
butenyl.

Under the term cyclic moiety is meant cyclohexan, and cyclopentane.

- 30 Under the term aromatic moiety is meant phenyl.

The wording "A and B forms a cyclic, heterocyclic or aromatic moiety" denotes cy-
clohexan, piperidine, benzene, and pyridine.

According to the invention two peptide sequences comprising two or more of the defined above amino acid sequences are connected to each other so that one of these two peptide sequences is covalently bound to one of two carboxylic group of a linker molecule selected from the molecules defined above and another of the peptide sequences is covalently bound to another carboxylic group of said linker molecule. The peptide sequences are covalently bound to the linker through their amino- or carboxy-groups of the N- or C terminal amino acid residue, respectively. Accordingly, a compound of the invention has the formula

5 COOH/CONH₂-peptide sequence-NH-CO-linker-CO-NH-peptide sequence-

COOH/CONH₂

10 or NH₂-peptide sequence-CO-NH-linker-NH-CO-peptide sequence-NH₂.

2. Production of the compound

15 2.1 Production of peptide fragments

The peptides of the present invention may be prepared by any conventional synthetic methods, recombinant DNA technologies, enzymatic cleavage of full-length proteins which the peptide sequences are derived from, or a combination of said 20 methods.

Recombinant preparation

Thus, in one embodiment the peptides of the invention are produced by use of recombinant DNA technologies.

25 The DNA sequence encoding a peptide or the corresponding full-length protein the peptide originates from may be prepared synthetically by established standard methods, e.g. the phosphoamidine method described by Beaucage and Caruthers, 1981, Tetrahedron Lett. 22:1859-1869, or the method described by Matthes et al., 1984, EMBO J. 3:801-805. According to the phosphoamidine method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in suitable vectors.

The DNA sequence encoding a peptide may also be prepared by fragmentation of the DNA sequences encoding the corresponding full-length protein of peptide origin, using DNase I according to a standard protocol (Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press, Cold Spring Harbor, NY, 1989). The present invention relates to full-length proteins selected from the groups of proteins identified above. The DNA encoding the full-length proteins of the invention may alternatively be fragmented using specific restriction endonucleases. The fragments of DNA are further purified using standard procedures described in Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press, Cold Spring Harbor, NY, 1989.

The DNA sequence encoding a full-length protein may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the full-length protein by hybridisation using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., 1988, Science 239:487-491.

The DNA sequence is then inserted into a recombinant expression vector, which may be any vector, which may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding a peptide or a full-length protein should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the coding DNA sequence in mammalian cells are the SV 40 promoter (Subramani et

al., 1981, Mol. Cell Biol. 1:854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., 1983, Science 222: 809-814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasudevam et al., 1992, FEBS Lett. 311:7-11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., 1980, J. Biol. Chem. 255:12073-12080; Alber and Kawasaki, 1982, J. Mol. Appl. Gen. 1: 419-434) or alcohol dehydrogenase genes (Young et al., 1982, in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al, eds., Plenum Press, New York), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., 1983, Nature 304:652-654) 5
promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., 1985, EMBO J. 4:2093-2099) or the 10
tpiA promoter.

15 The coding DNA sequence may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) 20
promoters. The vector may further comprise elements such as polyadenylation signals (e.g. from SV 40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV 40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

25 The recombinant expression vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV 40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hydromycin or methotrexate.

30 The procedures used to ligate the DNA sequences coding the peptides or full-length proteins, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

- To obtain recombinant peptides of the invention the coding DNA sequences may be usefully fused with a second peptide coding sequence and a protease cleavage site coding sequence, giving a DNA construct encoding the fusion protein, wherein the protease cleavage site coding sequence positioned between the HBP fragment and second peptide coding DNA, inserted into a recombinant expression vector, and expressed in recombinant host cells. In one embodiment, said second peptide selected from, but not limited by the group comprising glutathion-S-reductase, calf thymosin, bacterial thioredoxin or human ubiquitin natural or synthetic variants, or peptides thereof. In another embodiment, a peptide sequence comprising a protease cleavage site may be the Factor Xa, with the amino acid sequence *I*EGR, enterokinase, with the amino acid sequence *DDDDK*, thrombin, with the amino acid sequence *LVPR/GS*, or *Acharombacter lyticus*, with the amino acid sequence *XKX*, cleavage site.
- The host cell into which the expression vector is introduced may be any cell which is capable of expression of the peptides or full-length proteins, and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. *Xenopus laevis* oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the HEK293 (ATCC CRL-1573), COS (ATCC CRL-1650), BHK (ATCC CRL-1632, ATCC CCL-10) or CHO (ATCC CCL-61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159, 1982, pp. 601-621; Southern and Berg, 1982, J. Mol. Appl. Genet. 1:327-341; Loyter et al., 1982, Proc. Natl. Acad. Sci. USA 79: 422-426; Wigler et al., 1978, Cell 14:725; Corsaro and Pearson, 1981, in Somatic Cell Genetics 7, p. 603; Graham and van der Eb, 1973, Virol. 52:456; and Neumann et al., 1982, EMBO J. 1:841-845.
- Alternatively, fungal cells (including yeast cells) may be used as host cells. Examples of suitable yeast cells include cells of *Saccharomyces spp.* or *Schizosaccharomyces spp.*, in particular strains of *Saccharomyces cerevisiae*. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus spp.* or *Neurospora spp.*, in particular strains of *Aspergillus oryzae* or *Aspergillus niger*. The use of *Aspergillus spp.* for the expression of proteins is described in, e.g., EP 238 023.

5 The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements, or a suitable medium for growing insect, yeast or fungal cells. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

10 The peptides or full-length proteins recombinantly produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. HPLC, ion exchange chromatography, affinity chromatography, or the like.

15 Synthetic preparation

20 The methods for synthetic production of peptides are well known in the art. Detailed descriptions as well as practical advice for producing synthetic peptides may be found in Synthetic Peptides: A User's Guide (Advances in Molecular Biology), Grant G. A. ed., Oxford University Press, 2002, or in: Pharmaceutical Formulation: Development of Peptides and Proteins, Frokjaer and Hovgaard eds., Taylor and Francis, 1999.

25 Peptides may for example be synthesised by using Fmoc chemistry and with Acm-protected cysteins. After purification by reversed phase HPLC, peptides may be further processed to obtain for example cyclic or C- or N-terminal modified isoforms. The methods for cyclization and terminal modification are well-known in the art and described in detail in the above-cited manuals.

30 In a preferred embodiment the peptide sequences of the invention are produced synthetically, in particular, by the Sequence Assisted Peptide Synthesis (SAPS) method.

Sequence Assisted Peptide Synthesis (SAPS)

Peptides may be synthesised either batchwise in a polyethylene vessel equipped with a polypropylene filter for filtration or in the continuous-flow version of the polyamide solid-phase method (Dryland, A. and Sheppard, R.C., (1986) J.Chem. Soc. Perkin Trans. I, 125 - 137.) On a fully automated peptide synthesiser (Cameron ET al., (1987), J. Chem. Soc. Chem.Commun, 270-272) using 9-fluorenylmethyloxycarbonyl (Fmoc) or tert. -Butyloxycarbonyl, (Boc) as N-a-amino protecting group and suitable common protection groups for side-chain functionality's.

The following is a list of chemicals and a description of the procedure that may be helpful when using SAPS of the synthesis of the peptide fragments of the invention.

Chemicals and procedures

1. Solvents

DMF (N,N-dimethylformamide, Riedelde-Haen, Germany) (may be purified by passing through a column packed with a strong cation exchange resin, for example Lewatit S 100 MB/H strong acid, Bayer AG Leverkusen, Germany, and analysed for free amines prior to use by addition of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-Oli) giving rise to a yellow colour (Dhbt-O- anion) if free amines are present.

DCM (dichloromethane, analytical grade, Riedelde-Maen, Germany) may be used directly without purification.

2. Amino acids:

Fmoc-protected amino acids and corresponding pentafluorophenyl (Pfp) esters may be purchased from MilliGen, UK, NovaBiochem, Switzerland, and Bachem, Switzerland, and the Dhbt-esters from NovaBiochem, Switzerland in suitable side-chain protected forms.

Bocprotected amino acids may be purchased from Bachem, Switzerland.

30

3. Coupling reagents

Diisopropylcarbodiimide (DIC) may be purchased from Riedelde-Hen, Germany and distilled prior to use.

Dicyclohexylcarbodiimide (DCC) may be purchased from Merck-Schuchardt, Ni-inchen, Germany, and purified by distillation.O-Benzotriazoly-N,N,N",N"- tet-

ramethyluronium tetrafluoroborate (TBTU) (PerSeptive Biosystems GmbH Hamburg, Germany).

4. Linkers

- 5 HMPA, Novabiochem, Switzerland;
4-hydroxymethylbenzoic acid, Novabiochem;
4-methoxymandelic acid, Aldrich, Germany;
HMPB, Novabiochem; AM, Novabiochem;
3-(4-hydroxymethylphenoxy) propionic acid, Novabiochem, to be coupled to the
10 resin as a preformed I-hydroxybenzotriazole (HObt) ester generated by means of
DIC.
- Racemic 4-methoxymandelic acid (98% pure, Aldrich, Germany) may be used directly as linker or resolved by treatment with (+)-cinchonine (85% pure, Aldrich, Germany) giving the optical active linker (+)-4-methoxymandelic acid, $[\alpha]D20 = + 146$
15 (water) in 95.8 % optical purity and (-)-4-methoxymandelic acid, $[\alpha]D20 = - 128.6$ (water) in 88.1 % optical purity. Resolution of (+/-)-4-methoxymandelic acid (A. Mc Kenzie,D.J.C. Pirie, (1936), Berichte 69, 868; E. Knorr, (1904), Berichte 37, 3172). (+/-)-
4-Methoxymandelic acid (10 g, 54.89 mmol; Aldrich, 98%) is dissolved in 500 ml hot
water (60-80OC) and the solution decanted while still warm in order to remove in-
20 soluble impurities. (+)-Cinchonine (16,16 g, 54.89 mmol, Aldrich, 85%, $[\alpha]D20 = + 211$ (litt.: + 228.0)) is added to the hot solution in small portions. The solution be-
came clear after 15 min stirring at 60-80OC and is cooled in ice. After 1 h the pre-
cipitate is collected by filtration and dried in an exsiccator over night, yielding 9.9 g
25 of the chinconine salt. The salt is recrystallized from boiling water (80 ml); the solu-
tion decanted while still warm and then cooled in ice. The precipitate is collected by
filtration after 1h, washed three times with cold water, and dried in an exsiccator
over night yielding 7.84 g (16.45 mmol). The chinconine salt (2 g, 4.2 mmol) is dis-
solved in 40 ml 2NHC1 and immediately extracted with 3 x 30 ml diethylether. The
ether phase was dried over Na₂SO₄ and evaporated to dryness yielding 0.55 g 4-
30 methoxymandelic acid. The optical purity of the liberated 4methoxymandelic acid
was estimated to 18.5% ($[\alpha]D20 = + 270$). After a second recrystallisation of the
chinconine salt followed by liberation of the mandelic acid as described above the
optical purity was estimated to 69.0% ($[\alpha]D20 = + 100.8\cdot0$). A third recrystallisation
resulted in an optical purity of 95.8% ($[\alpha]D20 = + 140.0\cdot0$).

5. Solid supports

Peptides produced according to the Fmoc-strategy are synthesized on three different types of solid support using 0.05 M or higher concentrations of Fmoc-protected activated amino acid in DMF.

- 5 1) PEG-PS (polyethyleneglycol grafted on polystyrene; TentaGel S NH₂ resin, 0.27 mmol/g, Rapp Polymere, Germany or NovaSyn TG resin, 0.29 mmol/g, Novabiochem, Switzerland);
- 10 2) PepSyn Gel (polydimethylacrylamide resin functionalized with sarcosine methyl-ester, 1.0 mmol/g; MilliGen, UK).
- 10 3) PepSyn K (Kieselguhr supported polydimethylacrylamide resin functionalized with sarcosine methylester 0.11mmol/g; MilliGen, UK).

Peptides synthesised according to the Boc-strategy may be synthesised on a Merrifield-resin (polystyrenedivinylbenzene) with the first amino acid attached (Novabiochem, Switzerland).

15

6. Catalysts and other reagents

Diisopropylethylamine (DIEA) may be purchased from Aldrich, Germany,

Ethylenediamine from Fluka, Switzerland,

Piperidine from Riedel-deWen, Frankfurt, Germany.

20

4-(N,N-dimethylamino)pyridine (DMAP) may be purchased from Fluka, Switzerland and used as a catalyst in coupling reactions involving symmetrical anhydrides.

3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzo-triazine (Dhbt-OH) may be obtained from Fluka, Switzerland,

I-hydroxybenzotriazole (HObt) from NovaBiochem, Switzerland.

25

7. Coupling procedures

The first amino acid is coupled as a symmetrical anhydride in DMF generated from the appropriate N-cc-protected amino acid and DIC. The following amino acids are coupled as Pfp- or Dhbt-esters or as preformed HOBT esters made from appropriate

30

N-protected amino acids and HOBT by means of DIC or TBTU in DMF. In the case of Fmoc all acylations are checked by the ninhydrin test performed at 80OC in order to prevent Fmoc deprotection during the test.

8. Deprotection of the N-terminal amino acid-amino (N-a-amino) protecting group.

- Deprotection of the Fmoc group is performed by treatment with 20% piperidine in DMF (1x3 and 1x7 min when synthesized batchwise) or by flowing the deprotection solvent through the resin (10 min, flow rate 1 ml/min using continuous flow synthesis), followed by wash with DMF until no yellow colour (Dhbt-O-) could be detected
5. after addition of Dhbt-OH to the drained DMF. Deprotection of the Boc group is performed by treatment with TFA in DCM 10x1.5 min and 1x20 min followed by wash 6x9 min each with DCM, neutralisation with 10% triethylamine in DCM (v/v) 2x1.5 min each, followed by 6x9 min wash with DCM.
10. 9. Cleavage of peptide from resin with acid.
Peptides are cleaved from the resins by treatment with 95% trifluoroacetic acid (TFA, Halocarbon Products Corporation, U.S.A.; Biesterfeld & Co. Hamburg, Germany)-water v/v at r.t. for 2 h. The filtered resins are washed with 95% TFA-water and filtrates and washings evaporated under reduced pressure. The residue is
15. washed with ether and freeze dried from acetic acid-water. The crude freeze dried product is analysed by high-performance liquid chromatography (HPLC) and identified by matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI TOF MS) or by electrospray ionisation mass spectrometry (ES-MS).
20. 10. Cleavage of peptide from resin with base.
The dried resin (1 g) is treated with 1M sodium hydroxide (10 ml) at 4 °C and left for 15 min at room temperature. The resin is filtered into a flask containing 10% aq. acetic acid. The peptide is isolated by lyophilization and submitted to gel filtration.
25. 11. Cleavage of peptide from resin with TFMSA.
The dried resin (250 mg) is placed in a round-bottomed flask with a stirring bar. Thioanisole/ethanedithiol (2:1, 750KII) is added, the mixture chilled in ice, 5 ml TFA is added and the mixture is stirred for 5 - 10 min. TFMSA (500KII) is added drop wise and the reaction continued at room temperature (r.t.) for 30 - 60 min. The peptide is precipitated after addition of ether.
30. 12. Deprotection of side chain protective groups
Preferably, the side chains are deprotected simultaneously with the cleavage of the peptide from the resin.

13. Preformed HObt-ester**Method a.**

3 eq. N-a-amino protected amino acid is dissolved in DMF together with 3 eq. HObt

and 3 eq DIC. The solution was left at r.t. for 10 minutes and then added to the

- 5 resin, which had been washed with a solution of 0.2 Dhbt-OH in DMF prior to the addition of the preactivated amino acid.

Method b.

3 eq. N-a-amino protected amino acid is dissolved in DMF together with 3 eq. HObt,

- 10 3 eq TBTU and 4,5 eq. DIEA. The solution is left at r.t. for 5 minutes and then added to the resin. Preformed symmetrical anhydride 6 eq.N-u-amino protected amino acid is dissolved in DCM and cooled to 0 °C. DCC (3 eq.) is added and the reaction con-

tinued for 10 min. The solvent is removed in vacuum and the remainders dissolved in DMF. The solution is filtered and immediately added to the resin followed by 0.1 eq.

- 15 of DMAP.

14. Estimation of the coupling yield of the first N-a-amino protected amino acid

3 - 5 mg dry Fmoc-protected peptide-resin is treated with 5 ml 20 times piperidine in DMF for 10 min at r.t. and the UV absorption for the dibenzofulvenepiperidine ad-

- 20 duct is estimated at 301 nm. The yield is determined using a calculated extension coefficient e 301 based on a Fmoc-Ala-OH standard. In case of Boc-protection, the coupling is estimated according to the ninhydrin-method after removal of the Boc-group (Sarin, V.K. et al., (1981), Anal. Biochem, 117, 147-157).

25 15. Peptide synthesis on PepSyn K resin

Dry PepSyn K (ca 500 mg) is covered by ethylenediamine and left at rt over night.

The resin is drained and washed with DMF 10 x 15 ml, 5 min each. After draining the resin is washed with 10% DIEA in DMF v/v (2 x 15 ml, 5 min each) and finally washed with DMF until no yellow colour could be detected by addition of Dhbt-OH to

- 30 the drained DMF. 3 eq. HMPA 3 eq. HObt and 3 eq. DIC is dissolved in 10 ml DMF and left for activation for 10 min, after which the mixture is added to the resin and the coupling continued for 24 h. The resin is drained and washed with DMF (10 x 15 ml, 5 min each), and the acylation was checked by the ninhydrin test. The first amino acid is coupled as the side chain protected preformed symmetrical anhydride (see above), and the coupling yields estimated as described above. It is in all cases

to be better than 70%. The synthesis is then continued either as "continuous-flow" or as "batchwise" as described below.

16. Continued peptide synthesis on PepSyn K using continuous-flow technique.

- 5 The resin (ca. 500 mg) with the first amino acid attached is placed in a column connected to the fully automated peptide synthesiser. The Fmoc group is deprotected as described above. The remaining amino acids according to the sequence are coupled as Fmocprotected, if necessary side chain protected, Pfp esters (3 eq.) with the addition of Dhbt-OH (1 eq.). The end-point of each coupling is determined automatically by monitoring the disappearance of the yellow colour of the Dhbt-OH anion spectrophotometrically. After completed synthesis the peptide-resin is washed with DMF (10 min flow rate 1 ml/min), DCM (3x5 ml, 3 min each) and finally diethyl ether (3x5 ml each) removed from the column and dried in vacuum.

15 17. Continued batchwise peptide synthesis on PepSyn K.

- The resin (ca. 500 mg) with the first amino acid attached is placed in a polyethylene vessel equipped with a polypropylene filter for filtration, and the Fmoc-group deprotected as described above. The remaining amino acids according to the sequence are coupled as preformed Fmoc-protected, if necessary side chain protected, HOBr esters (3 eq.) in DMF (5 ml) prepared as described above. The couplings are continued for 2 h unless otherwise specified. Excess reagent is then removed by DMF washing (12 min, flow rate 1 ml/min). All acylations are checked by the ninhydrin test performed at 80 °C. After completed synthesis the peptide-resin is washed with DMF (10 min, flow rate 1ml/min), DCM (5x5 ml, 1 min each) and finally with diethyl ether (5x5 ml, 1 min each), and dried in vacuum.

18. Batchwise peptide synthesis on PEG-PS.

- TentaGel S NH₂ or NovaSyn TG resin (250 mg, 0.27-0.29 mmol/g) is placed in a polyethylene vessel equipped with a polypropylene filter for filtration. The resin is swelled in DMF (5 ml), and treated with 20% piperidine in DMF to secure the presence of nonprotonated amino groups on the resin. The resin is drained and washed with DMF until no yellow color could be detected after addition of Dhbt-OH to the drained DMF. HMPA (3 eq.) is coupled as a preformed HOBr-ester as described above and the coupling is continued for 24 h. The resin is drained and washed with DMF (5 x 5 ml, 5 min each) and the acylation checked by the ninhydrin test. The first

amino acid is coupled as a preformed symmetrical anhydride as described above. The coupling yields of the first Fmoc-protected amino acids are estimated as described above. It is in all cases to be better than 60%. The following amino acids according to the sequence are coupled as preformed Fmoc-protected, if necessary side chain protected, HOBr esters (3 eq.) as described above. The couplings are continued for 2 h. The resin is drained and washed with DMF (5 x 5 ml, 5 min each) in order to remove excess reagent. All acylations are checked by the ninhydrin test performed at 80 °C. After completed synthesis the peptide-resin is washed with DMF (3x5 ml, 5 min each), DCM (3x5 ml, 1 min each) and finally diethyl ether (3x5 ml, 1 min each) and dried in vacuo.

19. Batchwise peptide synthesis on PepSyn Gel

Dry PepSyn Gel resin (500 mg, 1 mmol/g) is placed in a polyethylene vessel equipped with a polypropylene filter for filtration. The resin is swelled in ethylenediamine (15 ml) and gently agitated by shaking for 20 h. The resin is drained and washed with DMF (10 x 15 ml, 5 min each). After draining the resin is washed with 10% DIEA in DMF v/v (2 x 15 ml, 5 min each) and finally washed with DMF (5 x 15 ml, 5 min each) until no yellow colour could be detected after addition of Dhbt-OH to the drained DMF. HMPA (3eq.) is coupled as a preactivated HOBr-ester as described above (method a) and the coupling is continued for 24 h. The resin is drained and washed with DMF (5 x 15 ml, 5 min each). The acylation is checked by the ninhydrin test. The first amino acid is coupled as preformed side chain protected symmetrical anhydride as described above. The coupling yields of the first Fmoc-protected amino acids are estimated as described above. It is in all cases to be better than 70%. The remaining amino acids according to the sequence are coupled as preformed Fmoc-protected, if necessary side chain protected, HOBr esters (3 eq.) as described above (method a). The couplings are continued for 2 h and, if necessary, double coupled over night. The resin is drained and washed with DMF (5 x 5 ml, 5 min each) in order to remove excess reagent. All acylations are checked by the ninhydrin test performed at 80 °C. The Fmoc group is deprotected as described above. After completed synthesis the peptide-resin is washed with DMF (3x15 ml, 5 min each), DCM (3x15 ml, 2 min each) and finally diethyl ether (3x15 ml, 2 min each) and dried in vacuum.

35 19 HPLC.

HPLC may be performed on a Waters 600 E instrument equipped with a Waters 996 Photodiode array detector with a Waters Radial Pak 8 x 100 mm C18 reversed-phase column. Buffer A is 0.1 vol % TFA in water, and buffer B is 90 vol % acetonitrile, 9.9 vol % water and 0.1 vol % TFA. The Buffers are pumped through the column at a flow rate of 1.5 ml/min using the following gradient: linear gradient from 0% - 70% B (20 min), linear gradient from 70 - 100% B (1 min), isocratic 100% B (5 min). 2. Isocratic with 0% B (2 min), linear gradient from 0 - 50% B (23 min), linear gradient from 50 - 100% B (5 min), isocratic 100% B (5 min).

10 **2.2 Production of the compound**

According to the invention a compound of interest is preferably obtained by the LPA method:

LPA method

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The LPA method is disclosed in WO 00/18791. The method essentially comprises the following steps:

- (a) providing by solid phase synthesis or fragment coupling peptide fragment(s) comprising the desired sequence(s), the peptide fragment(s) being attached to a solid phase;
 - (b) if necessary, deprotecting any N-terminal amino groups while the peptide fragment(s) are still attached to a solid phase,
 - (c) reacting the peptide fragment(s) having unprotected N-terminal groups with an achiral di-, tri-, or tetracarboxylic acid so as to provide a construct having a ring structure, and
 - (d) cleaving the construct from the solid phase so as to provide an LPA comprising the peptide fragment(s) having free C-terminal groups.
- 30 In the above method, prior step (d) the following steps may be performed:
- (c1) if present, deprotecting any N-protected groups originating from the carboxylic acid used in step (c),
 - (c2) continuing the solid phase synthesis or fragment coupling so as to provide peptide fragment(s) comprising desired sequence(s) having at least one N-protected N-terminal amino acid group and/or attaching chemical moieties, and

(c3) deprotecting, if present, any N-terminal amino groups (prior or after step (d)).

The method provides i.a. LPAs presenting desired sequences of the invention with N to C orientation (step (c)). And also simultaneously sequences with C to n orientation (step (c2))

Thus, to obtain a compound of the invention two peptide chains attached to a solid phase are to be assembled by means of achiral di-, tri- or tetracarboxylic acids. Suitable achiral di-, tri- or tetracarboxylic acids to be used in the present method have the general formula



wherein n and m independently are an integer of from 1 to 20, X is HN, A and B independently are a substituted or unsubstituted C_{1-10} alkyl, a substituted or unsubstituted C_{2-10} alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety, or A and B together form a substituted or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

In another embodiment suitable achiral di-, tri- or tetracarboxylic acids to be used in the present method have the general formula



wherein n and m are 0 or an integer of from 1 to 20, X is $H_2N(CR_2)pCR$, or $RHN(CR_2)pCR$, wherein p is 0 or integer of from 1 to 20, wherein each R is H, a substituted or unsubstituted C_{1-10} alkyl, a substituted or unsubstituted C_{2-10} alkenyl, a substituted or unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety, or A and B together form a substituted or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

In still another embodiment suitable achiral di-, tri- or tetracarboxylic acids to be used in the present method have the general formula

X[(A)nCOOH][(B)mCOOH]

wherein n and m are 0 or an integer of from 1 to 20, X is HO(CR₂)pCR,
5 HS(CR₂)pCR, halogen-(CR₂)pCR, HOOC(CR₂)pCR, ROOC(CR₂)pCR,
HCO(CR₂)pCR, RCO(CR₂)pCR, or [HOOC(A)n][HOOC(B)m]CR(CR₂)pCR, wherein
p is 0 or integer of from 1 to 20, each R independently is H or a substituted or un-
substituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or
10 unsubstituted cyclic moiety, a substituted or unsubstituted heterocyclic moiety, a
substituted or unsubstituted aromatic moiety, or A and B together form a substituted
or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, sub-
stituted or unsubstituted aromatic moiety.

In yet another embodiment suitable achiral di-, tri- or tetracarboxylic acids to be
used in the present method have the general formula

15

X[(A)nCOOH][(B)mCOOH]

Wherein n and m are 0 or an integer of from 1 to 20, X is H₂N(CR₂)p, RHN(CR₂)p,
HO(CR₂)p, HS(CR₂)p, halogen-(CR₂)p, HOOC(CR₂)p, ROOC(CR₂)p, HCO(CR₂)p,
20 RCO(CR₂)p, or [HOOC(A)n][HOOC(B)m](CR₂)p, wherein p is 0 or integer of from 1
to 20, each R independently is H or a substituted or unsubstituted C₁₋₁₀ alkyl, a sub-
stituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety, a
substituted or unsubstituted heterocyclic moiety, a substituted or unsubstituted aro-
matic moiety, or A and B together form a substituted or unsubstituted cyclic moiety,
25 substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aro-
matic moiety.

Under the term C₁₋₁₀ alkyl is meant straight or branched chain alkyl groups having 1-
10 carbon atoms, e.g. methyl, ethyl, isopropyl, butyl, and tertbutyl.

30

Under the term C₂₋₁₀ alkenyl is meant straight or branched chain alkenyl groups
having 2-10 carbon atoms, e.g. ethynyl, propenyl, isopropenyl, butenyl, and tert-
butenyl.

35

Under the term cyclic moiety is meant cyclohexan, and cyclopentane.

Under the term aromatic moiety is meant phenyl.

5 The wording "A and B forms a cyclic, heterocyclic or aromatic moiety" denotes cyclohexan, piperidine, benzene, and pyridine.

By reaction with a carboxylic acid, a construct of the type

X(CO-sequence)2-solid phase, wherein X as defined above,

10 is obtained.

15 By the term "sequence" is in the present content meant a peptide comprising naturally occurring and/or non-naturally occurring amino acids, a PNA-sequence, or peptidomimetic. By "naturally occurring amino acids" is meant L- and D-forms of the 20 acids found in nature. Non-naturally occurring amino acids are e.g. modified naturally occurring amino acids. The term sequence is further intended to comprise one or more of such sequences. Examples of suitable peptidomimetics are described in Marshall G.R.,(1993) Tetrahedron, 49:3547-3558. The term "chemical 20 moieties" denotes an entity enhancing the solubility or biological activity of the LPA, and entity for directing the LPA to its target or a marker. Preferred embodiments for the sequences are described above.

25 The group X permits directly or indirectly continued stepwise synthesis or a fragment coupling of the same sequence, or of one or more different sequences and/or moieties. Orientation of peptide fragments (N to C or C to N) in LPA is defined as desired. One embodiment of the present invention features LPAs with N to C orientation, another embodiment concerns simultaneous N to C and C to N presentation of the sequences, and yet another embodiment the sequences have C to N orientation.

30 In the case where X comprises a temporally protected amino function, synthesis or coupling can be carried out directly after protection. Suitable activation of all carboxyl-containing groups providing effective formation of the ring system (on step (c), see above) can be ensured using half-equivalent carboxy acid. In case of tri- or tetra-carboxylic acids the activated carboxy group may further be derivatised with a

diamine such as ethylenediamine or an amine suitably functionalised for further reactions such as mercapto-, an oxy-, an oxo or carboxyl group. In the case of diamine, peptide synthesis or fragment coupling can be continued directly according to the desired sequence or chemical moiety. In a preferred embodiment, the Fmoc-protection strategy is used, but any amino protection group may be used depending on the synthesis or coupling strategy. Examples are the Boc-protection group strategy.

Since the continued stepwise synthesis or fragment coupling is performed with one or in case of a bifunctional chemical moiety such lysine with two amino acid groups, it has surprisingly been found that a much better result can be obtained as compared to conventional tetrameric lysine dendimers obtained by the MAP synthesis. Furthermore, optimal peptide synthesis procedures or coupling procedures can be used for the single chains attached to the solid phase, and their homogeneity can be verified prior to forming the LPA. Cleavage from the solid phase and simultaneous side-chain deprotection can be performed by standard peptide synthesis procedures (described above). A final product may thus be obtained having optimal and well-defined composition. Purification by standard chromatography methods such as HPLC or gel filtration can be easily be performed, if desired or needed.

Favourable di-, tri- and tetracarboxylic acids for providing the ring structure may for example be selected from imino diacetic acid, 2-amino malonic acid, 3-amino glutaric acid, 3-methylamino glutaric acid, 3-chloro glutamic acid, 3-methoxy-carbonyl glutaric acid, 3-acetyl glutaric acid, glutaric acid, tricarballylic acid, 3,4-bis-carboxymethyl adipic acid, 4-(2-carboxyethyl)-pimelic acid, (3,5-bis-carboxymethyl-phenyl)-acetic acid, 3,4-bis-carboxymethyl-adipic acid, benzene-1,2,4,5-tetra carboxylic acid, 4-(3-carboxy-allylamino)-but-2-enoic acid, 4,4-imino-dibenzoic acid, 1,4-dihydropyridine-3,5-dicarboxylic acid, 5-amino isophthalic acid, 2-chloro malonic acid, 3-hydroxy glutaric acid, and benzene-1,3,5-tricarboxylic acid.

Fragment coupling (fragment coupling or fragment condensation) may be performed according to standard procedures, e.g. as described in Peptide Synthesis protocols, Methods in Molecular Biology Vol. 35, Chapter 15, 303-316, Nyfeler R, Pennington MW and Dunne BM Eds., Humana Press, 1994. Accordingly, fragments may be synthesised on a solid phase, cleaved from the solid phase with full preservation of

protecting groups, purified and characterised as described above. Suitable fragments may also be obtained by other techniques described above.

It is a preferred embodiment of the invention to use the above LPA method for the
5 production of a compound of the invention.

3. Biological activity

According to the invention a compound of the invention can bind and modulate ac-
10 tivity of a cell surface receptor through a binding site located within the peptide se-
quence(s) of the compound, said binding site being defined by the presence in the
binding site structure the amino acid sequence of the formula

L1-A-L2-B-L3-C-L4-D-L5

wherein

15 one of A, B, C, D is selected from a hydrophobic amino acid residue,
one of A, B, C, D is selected from a basic amino acid residue or Ser, Thr, Asn or
Gln,
one of A, B, C, D is selected from an acidic amino acid residue or Ser, Thr, Asn
or Gln,
20 one of A, B, C, D is Gly or Ala, and
L1, L2, L3, L4 and L5 is selected from a chemical bond or an amino acid se-
quence having n amino acid residues, wherein n is an integer of from 0 to 5.

Therefore, all peptide sequences having a length of 4 to 80 amino acid residues
25 comprising the above receptor binding structural motif are in the scope of the inven-
tion. Preferred embodiments of the peptide sequences are described above. The
present invention in a preferred embodiment features a compound comprising at
least two sequences, each of said two sequences being capable of binding to at
least one receptor molecule, wherein both sequences comprising the above struc-
30 tural motif.

Receptor

Thus, a compound of the invention is capable of binding of a receptor, in particular a
35 cell-surface receptor.

A cell-surface receptor is defined in the content of the present invention as any molecule comprising at least one polypeptide chain, said molecule being associated with the plasma membrane of a cell in such a way that enables said molecule to receive a signal at the outer side of the membrane, transduce the signal through the membrane, and convey said signal further by inducing a certain action on molecular level inside the cell, for example by inducing association or dissociation of molecular complexes, or initiating a biochemical reaction, for example auto-phosphorylation of the receptor, or proteolytic cleavage of the receptor leading to initiation of intracellular signal transduction.

The invention designates to a "functional cell-surface receptor". "Functional cell-surface receptor" is meant that the cell-surface receptor of the invention has an identifiable group of ligands, the binding of these ligands to the receptor induces intracellular signal transduction, which results in a physiological response of the cell. The physiological response, such as for example differentiation, proliferation, survival, apoptosis or motility of a cell, depends on the ligand that is involved in the interaction with the receptor, and/or characteristics of said interaction, such as the affinity or duration, and/or a species of the cell which expresses the receptor. By the term "ligand" is defined a compound which is capable to bind to the receptor and thereby to activate or inhibit said receptor. "Activation" of a receptor is meant that after extracellular binding of a ligand the receptor became capable to transmit the effect of "ligand binding" into a cascade of biochemical reactions collectively termed "receptor signalling" or "signal transduction" inside the cell resulting in one of the above mentioned physiological responses of the cell. "Inhibition" of a receptor is meant that after extracellular binding of a ligand the receptor became "silent" or "inactive" and is not capable anymore initiate a cascade of biochemical reactions which is normally initiated in response to receptor ligand binding. According to the invention compounds featured above are capable of activation or inhibition of a functional cell-surface receptor.

The functional cell-surface receptor of the invention, is in a preferred embodiment a receptor of the family of fibroblast growth factor receptors (FGFRs) comprising FGFR1, FGFR2, FGFR3 and FGFR4. In the most preferred embodiment a cell-surface receptor of the invention is FGFR1 or a functional homologue thereof.

By the term "functional homologue of the receptor" is meant a molecule which is capable of

- 5 i) extracellular binding of a ligand of FGFR and thereby activating the receptor dependent signal transduction cascade in the cell, and/or
ii) intracellular binding of an adapter molecule of FGFR and thereby activating the receptor dependent signal transduction cascade in the cell.

10 The term "binding" refers to a direct or indirect interaction between an FGFR homologue and a counter molecule having a binding site for FGFR. In the present context the counter molecule is an extracellular ligand of FGFR, or an intracellular adapter molecule. An "adapter molecule" is defined in the content of the invention as a molecule, which is capable to recognise an "active state" of the receptor, selectively bind to such receptor and convey the signal of "activated receptor" in the 15 cell by inducing a cascade of reactions of signal transduction. An intracellular adapter molecule may be represented by for example STN, FRS, Grb, SHP2, PLC γ , or PIP3.

20 Activating of a receptor often depends on the receptor dimerisation induced by ligand binding. Therefore, a capability of a compound to promote clustering or dimerisation (multimerisation) of the receptor molecules would be advantageous. Thus, in one embodiment the invention features a compound that is capable of activating of a receptor. Such activating of a receptor may take place for example in situation when a natural ligand of the receptor is absent and the receptor is temporary "silent", inactive. From another side, synthetic compounds that are capable of binding to a receptor may compete with a natural ligand for the receptor binding or prevent a natural ligand to bind to the receptor and thereby said synthetic compounds may modulate the receptor signalling. The later may takes place when a natural ligand and a synthetic compound have different affinity of binding to the receptor. It has been 25 shown in the art that the cellular response to activation of a receptor depends on the strength of receptor stimulation, which may, for example, be characterised by the value of affinity of interaction of the receptor with a ligand, and/or by the duration of such interaction. The both affinity and duration of interaction of a receptor and the receptor natural ligand may be affected if the interacting molecules are exposed to a 30 compound capable of binding to the receptor, such as for example a compound of
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the invention. Thus, it is another embodiment of the invention to provide a compound, which is capable of modulating the receptor signalling. According to the invention the modulating may result as in enhancing a physiologic response taking place when the receptor signalling is activated, as in inhibiting a physiologic response taking place when the receptor signalling is inhibited. Accordingly, the invention is still another embodiment related to a compound capable of inhibiting the receptor signalling.

Affinity of interaction between the compound and the receptor

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By the term "interaction" is meant that a compound has a transient or permanent direct or indirect contact with a cell-surface receptor.

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Any interaction between two molecules may be characterised by its affinity, which means the strength of attraction between two molecules. The affinity of interaction is commonly expressed by a value of K_d , a dissociation equilibrium constant. K_d reflects a ratio between the rate of dissociation and the rate of binding between two molecules and thus represents a measure of the strength of binding between two molecules. The stronger the binding, the lower is the value of K_d .

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The invention relates to compounds having a relatively low affinity binding to FGFR. The low affinity interaction of the invention is characterised by K_d having a value in the range of 10^{-3} to 10^{-10} , such for example in the range of 10^{-4} to 10^{-8} .

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Modulation of biological function of the receptor

A capability of a cell-surface receptor to induce and/or maintain a cellular process upon the ligand binding is herein termed "biological function" of the receptor.

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The invention features compounds that are capable of modulating of biological function of the receptor by modulating receptor signalling. By "modulating receptor signalling" is meant activation or inhibition of the production of a cascade of messenger molecules, which normally takes place in the cell in response to activation of the receptor by an extracellular stimulus.

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The receptor signalling leads to a physiological response of the cell. Therefore, by modulating receptor signalling it is possible to modulate a physiological response of the cell.

- 5 The invention preferably concerns an FGFR1-associated signalling, which may be reflected by i) the degree of FGFR1 tyrosine phosphorylation; ii) the activation state of one or more intracellular proteins involved in any of the FGFR-associated signal transduction pathways, such as for example the STAT1, JNK, PLC γ , ERK, STAT5, PI3K, PKC, FRS2 and/or GRB2 proteins; and/or iii) a cellular response.

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When concerned the FGFR signalling dependent cellular response, the invention relates to a cellular response selected but not limited from cell differentiation and/or cell dedifferentiation, apoptosis and/or cell survival, cell motility, cell proliferation.

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Medicament

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Cell death plays a key role in normal neuronal development, where 50% of the developing neurons are eliminated through programmed cell death, and in the pathophysiology of neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases. FGFRs and their ligands has been shown to be important determinants of neuronal survival both during development and during adulthood (Reuss and von Bohlen und Halbach (2003) Cell tissue Res, 313:139-57). Therefore, a compound, which is capable to promote neuronal cell survival by binding and activation FGFR is highly desirable. Thus, in one aspect the invention features compounds that promote survival of neural cells and can be used as medicaments for the treatment of conditions involving neural cell death. However, a compound of the invention may also be used as a medicament for promotion of survival of another type of cells, e.g. different type of muscle cells, or, alternatively, for enhancing cell death of still another type of cells, e.g. cancer cells, as the FGFR signalling has been shown to be a survival factor for both muscle and cancer cells (Ozen et al. (2001) J Nat Cancer Inst. 93:1783-90; Miyamoto et al. (1998) J Cell Physiol. 177:58-67; Detilliux et al. (2003) Cardiovasc Res. 57:8-19).

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Activity of cell-surface receptors is strictly regulated in a healthy organism. Mutations, abnormal expression or processing of a receptor or the receptor ligands lead to abnormalities in activity of the receptor and therefore lead to dysfunction of the

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receptor. The dysfunction of the receptor is in turn a reason for dysfunction of the cells which use the receptor for maintenance of various cellular processes. The latter is the manifestation of a disease. It has also been shown that attenuation of FGFR signalling leads to development of a number of different of pathologic conditions, e.g. diabetes (Hart et al., Nature 2000, 408:864-8). Activation of FGF receptors is involved as in normal, as in pathologic angiogenesis (Slavin, Cell Biol Int 1995, 19:431-44). It is important for development, proliferation, functioning and survival skeletal muscle cells, cardiomyocytes and neurons (Merle et al., J Biol Chem 1995, 270:17361-7; Cheng and Mattson, Neuron 1991, 7:1031-41; Zhu et al., Mech Ageing Dev 1999, 108:77-85). It plays a role in maintenance of normal kidney structure (Cancilla et al., Kidney Int 2001, 60:147-55), and it is implicated in wound healing and cancer disease (Powers et al., Endocr Relat Cancer. 2000, 7:165-97).

The present invention provides compounds capable of modulation of the activity of FGFRs. Consequently, said compounds are concerned by the invention as medicament for the treatment of diseases, wherein modulation of the activity of FGFRs may be considered as an essential condition for the curing.

Thus, the medicament of the invention is in one embodiment for prevention and/or treatment of

- 20 1) diseases and conditions of the central and peripheral nervous system, or of the muscles or of various organs, and/or
- 25 2) diseases or conditions of the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression; for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel, and/or

- 3) postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression, and/or
- 5 4) cancer disease, and/or
- 5) prion diseases.

10 The invention concerns the cancer being any type of solid tumors requiring neoangiogenesis.

15 The invention concerns prion diseases selected from the group consisting of scrapie, Creutzfeldt-Jakob disease. It has been shown that FGFRs plays a distinct role in prion diseases (Castelnau et al. (1994) *Exp Neurobiol.* 130:407-10; Ye and Carp (2002) *J Mol Neurosci.* 18:179-88).

- In another embodiment a compound of the invention is for the manufacture of a medicament for
- 20 1) promotion of wound-healing, and/or
- 2) prevention of cell death of heart muscle cells, such as after acute myocardial infarction, or after angiogenesis, and/or
- 3) revascularisation, and/or
- 4) stimulation of the ability to learn and/or of the short and/or long-term memory.

25 In still another embodiment a compound of the invention is for the manufacture of a medicament for

1) prevention of cell death due to ischemia;

2) prevention of body damages due to alcohol consumption;

30 In yet still another embodiment a compound of the invention is for manufacture of a medicament for the treatment of normal, degenerated or damaged cells which normally in vivo express one or more of the proteins selected from the group consisting

35 Neural Cell Adhesion Molecule (NCAM) (Swiss-Prot Ass. Nos: P13591,

- P13595-01, P13595),
- Neural cell adhesion molecule L1 (Swiss-Prot Ass. Nos: Q9QYQ7, Q9QY38, P11627, Q05695, P32004),
 - Neural Cell Adhesion Molecule-2 (NCAM-2) (Swiss-Prot Ass. No: P36335)
- 5 - Neuron-glia Cell Adhesion Molecule (Ng-CAM) (Swiss-Prot Ass. No: Q03696; Q90933),
- Neural cell adhesion molecule CALL (Swiss-Prot Ass. No: O00533),
 - Neuroglian (Swiss-Prot Ass. No: P91767, P20241),
 - Nr-CAM (HBRAVO, NRCAM, NR-CAM 12) (Swiss-Prot Ass. Nos: Q92823, O15179, Q9QVN3
- 10 - Axonin-1/TAG-1 (Swiss-Prot Ass. Nos: Q02246, P22063, P28685),
- Axonal-associated Cell Adhesion Molecule (AxCAM) (NCBI Ass. No: NP_031544.1; Swiss-Prot Ass. No: Q8TC35),
 - Myelin-Associated Glycoprotein (MAG) (Swiss-Prot Ass. No: P20917),
- 15 - Neural cell adhesion molecule BIG-1 (Swiss-Prot Ass. No: Q62682),
- Neural cell adhesion molecule BIG-2 (Swiss-Prot Ass. No: Q62845),
 - Fasciclin (FAS-2) (Swiss-Prot Ass. No: P22648),
 - Neural cell adhesion molecule HNB-3/NB-3 (Swiss-Prot Ass. Nos: Q9UQ52, P97528, Q9JMB8)
- 20 - Neural cell adhesion molecule HNB-2/NB-2 (Swiss-Prot Ass. Nos: O94779, P07409, P97527),
- Cadherin (Swiss-Prot Ass. No: Q9VW71),
 - Junctional Adhesion Molecule-1 (JAM-1) (Swiss-Prot Ass. Nos: Q9JKD5, O88792),
- 25 - Neural cell adhesion F3/F11(Contactin) (Swiss-Prot Ass. Nos: Q63198, P1260, Q12860, Q28106, P14781, O93250),
- Neurofascin (Swiss-Prot Ass. Nos: Q90924, Q91Z60; O42414),
 - B-lymphocyte cell adhesion molecule CD22 (Swiss-Prot Ass. Nos: Q9R094, P20273),
- 30 - Neogenin (NEO1) (Swiss-Prot Ass. Nos: Q92859, P97603, Q90610, P97798),
- Intercellular Cell Adhesion Molecule-5 (ICAM-5/telencephalin) (Swiss-Prot Ass. Nos: Q8TAM9, Q60625) or
 - Galactose binding lectin-12 (galectin-12) (Swiss-Prot Ass. Nos: Q91VD1, Q9JKX2, Q9NZ03),
- 35 - Galactose binding lectin-4 (galectin-4) (Swiss-Prot Ass. No: Q8K419; P38552).

- Fibroblast Growth Factor Receptor 1 (FGFR1) (Swiss-Prot Ass. Nos: Q9QZM7, Q99AVV7, Q9UD50, Q63827),
- Fibroblast Growth Factor Receptor 2 (FGFR2) (Swiss-Prot Ass. Nos: Q96KM2, P21802, Q63241),
- 5 - Fibroblast Growth Factor Receptor 3 (FGFR3) (Swiss-Prot Ass. Nos: Q95M13, AF487554, Q99052),
- Fibroblast Growth Factor Receptor 4 (FGFR4) (Swiss-Prot Ass. No: Q91742),
- Neurotrophin Tyrosin Kinase Type-2 (NTRKT-2) (Swiss-Prot Ass. No: Q8WXJ5),
- 10 - Leukocyte Antigen Related Protein-Tyrosine Phosphatase (LAR-PTPRF) (Swiss-Prot Ass. Nos: Q9EQ17, Q64605, Q64604, Q9QW67, Q9VIS8 P10586),
- Nephrin (Swiss-Prot Ass. Nos: Q925S5, Q9JIX2, Q9ET59, Q9R044, Q9QZS7, Q06500),
- 15 - Protein-Tyrosine Phosphatase Receptor type S (PTPRS) (Swiss-Prot Ass. Nos: Q64699, Q13332, Q75870),
- Protein-Tyrosine Phosphatase Receptor type kappa (R-PTP-kappa) (Swiss-Prot Ass. No: Q15262),
- Protein-Tyrosine Phosphatase Receptor type D (PTPRD) (Swiss-Prot Ass.
- 20 Nos: Q8WX65, Q9IAJ1, P23468, Q64487),
- Ephrin type-A receptor 8 (EPHA8/Tyrosine-Protein Kinase Receptor EEK) (Swiss-Prot Ass. Nos: O09127, P29322),
- Ephrin type-A receptor 3 (EPHA8/Tyrosine-Protein Kinase Receptor ETK-1/CEK4) (Swiss-Prot Ass. No: P29318),
- 25 - Ephrin type-A receptor 2 (Swiss-Prot Ass. No: Q8N3Z2)
- Insulin Receptor (IR) (Swiss-Prot Ass. No: Q9PWN6)
- Insulin-like Growth Factor-1 Receptor (IGF-1) (Swiss-Prot Ass. Nos: Q9QVW4, P08069, P24062, Q60751, P15127, P15208)
- Insulin-related Receptor (IRR) (Swiss-Prot Ass. No: P14616),
- 30 - Tyrosine-Protein Kinase Receptor Tie-1 (Swiss-Prot Ass. Nos: 06805, P35590, Q06806),
- Roundabout receptor-1 (robo-1) (Swiss-Prot Ass. Nos: O44924, AF041082, Q9Y6N7),
- Neuronal nicotinic acetylcholine receptor alpha 3 subunit (CHRNA3) (Swiss-
- 35 Prot Ass. Nos: Q8VHH6, P04757, Q8R4G9, P32297)

- Neuronal acetylcholine receptor alpha 6 subunit (Swiss-Prot Ass. Nos: Q15825, Q9R0W9),
 - Platelet-Derived Growth Factor Receptor Beta (PDGFRB) (Swiss-Prot Ass. Nos: Q8R406, Q05030),
 - Interleukin-6 Receptor (IL-6R) (Swiss-Prot Ass. No: Q00560),
 - Interleukin-23 Receptor (IL-23R) (Swiss-Prot Ass. No: AF461422),
 - Beta-common cytokine receptor of IL-3, IL5 and GmCsf (Swiss-Prot Ass. No: P32927)
 - Cytokine Receptor-Like molecule 3 (CRLF1) (Swiss-Prot Ass. No: Q9JM58),
 - Class I Cytokine Receptor (ZCYTOR5) (Swiss-Prot Ass. No: Q9UHH5)
 - Netrin-1 receptor DCC (Swiss-Prot Ass. No: P43146),
 - Leukocyte Fc Receptor-like Protein (IFGP2) (Swiss-Prot Ass. Nos: Q96PJ6, Q96KM2),
 - Macrophage Scavenger Receptor 2 (MSR2) (Swiss-Prot Ass. No: Q91YK7), or
 - Granulocyte Colony Stimulating Factor Receptor (G-CSF-R) (Swiss-Prot Ass. No: Q99062),
 - perlecan (Swiss-Prot Ass. No: P98160),
 - ADAM-8 (Swiss-Prot Ass. No: Q05910),
 - ADAM-19 (Swiss-Prot Ass. Nos: Q9H013, O35674),
 - ADAM-8 (Swiss-Prot Ass. No: P78325),
 - ADAM-12 (Swiss-Prot Ass. Nos: O43184, Q61824),
 - ADAM-28 (Swiss-Prot Ass. Nos: Q9JLN6, Q61824, Q9XSL6, Q9UKQ2),
 - ADAM-33 precursor (Swiss-Prot Ass. Nos: Q8R533, Q923W9),
 - ADAM-9 (Swiss-Prot Ass. Nos: Q13433, Q61072),
 - ADAM-7 (Swiss-Prot Ass. Nos: Q9H2U9, O35227, Q63180),
 - ADAM-1A Fertilin alpha (Swiss-Prot Ass. No: Q8R533),
 - ADAM-15 (Swiss-Prot Ass. Nos: Q9QYV0, O88839, Q13444),
 - Metalloproteinase-desintegrin domain containing protein (TECAM) (Swiss-Prot Ass. No: AF163291),
 - Metalloproteinase 1 (Swiss-Prot Ass. Nos: O95204, Q9BSI6),
 - Collagen type VII (Swiss-Prot Ass. No: Q63870),
 - Fibronectin (Swiss-Prot Ass. Nos: Q95KV4, Q95KV5, P07589, Q28377, U42594, O95609, P11276), or
 - Tenascin-R (Swiss-Prot Ass. Nos: Q15568, O00531, Q90995, P10039)
 - Cytokine-like factor-1 (CLF-1) (Swiss-Prot Ass. No: O75462),

- or a fragment, or a variant thereof.

In particular, the invention concerns normal, degenerated or damaged NCAM presenting cells.

5 According to invention the above listed protein are natural ligands of FGFR and a compound of the invention comprising at least two peptide fragments derived from the sequence of any of the above proteins, said fragments being capable of binding to FGFR and modulating FGFR signalling, may advantageously be used as a mimic of the corresponding protein.

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The medicament of the invention comprises an effective amount of one or more of the compounds as defined above, or a pharmaceutical composition comprising one or more compound of the invention and pharmaceutically acceptable additives.

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Thus, the invention in another aspect also concerns a pharmaceutical composition comprising at least one compound of the invention.

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A further aspect of the invention is a process of producing a pharmaceutical composition, comprising mixing an effective amount of one or more of the compounds of the invention, or a pharmaceutical composition according to the invention with one or more pharmaceutically acceptable additives or carriers.

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In one embodiment of the process as mentioned above, the compounds are used in combination with a prosthetic device, wherein the device is a prosthetic nerve guide. Thus, in a further aspect, the present invention relates to a prosthetic nerve guide, characterised in that it comprises one or more of the compounds or the pharmaceutical composition as defined above. Nerve guides are known in the art.

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The invention relates to the use a medicament and/or pharmaceutical composition comprising the compound of invention for the treatment or prophylaxis of any of the diseases and conditions mentioned below.

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Such medicament and/or pharmaceutical composition may suitably be formulated for oral, percutaneous, intramuscular, intravenous, intracranial, intrathecal, intracerebroventricular, intranasal or pulmonal administration.

- Strategies in formulation development of medicaments and compositions based on the compounds of the present invention generally correspond to formulation strategies for any other protein-based drug product. Potential problems and the guidance required to overcome these problems are dealt with in several textbooks, e.g. "Therapeutic Peptides and Protein Formulation. Processing and Delivery Systems", Ed. A.K. Banga, Technomic Publishing AG, Basel, 1995.
- 5
- Injectables are usually prepared either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. The preparation may also be emulsified. The active ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like, and combinations thereof. In addition, if desired, the preparation may contain 10 minor amounts of auxiliary substances such as wetting or emulsifying agents, pH-buffering agents, or which enhance the effectiveness or transportation of the preparation.
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- Formulations of the compounds of the invention can be prepared by techniques known to the person skilled in the art. The formulations may contain pharmaceutically acceptable carriers and excipients including microspheres, liposomes, micro-capsules, nanoparticles or the like.
- 20
- The preparation may suitably be administered by injection, optionally at the site, where the active ingredient is to exert its effect. Additional formulations which are suitable for other modes of administration include suppositories, nasal, pulmonal and, in some cases, oral formulations. For suppositories, traditional binders and carriers include polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of from 0.5% to 25 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and generally contain 10-95% of the active 30 35 ingredient(s), preferably 25-70%.

Other formulations are such suitable for nasal and pulmonal administration, e.g. inhalators and aerosols.

- 5 The active compound may be formulated as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide compound) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic acid, oxalic acid, tartaric acid, mandelic acid, and the like. Salts formed with the free carboxyl group may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.
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- 15 The preparations are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated, including, e.g. the weight and age of the subject, the disease to be treated and the stage of disease. Suitable dosage ranges are per kilo body weight normally of the order of several hundred µg active ingredient per administration with a preferred range of from about 0.1 µg to 5000 µg per kilo body weight. Using monomeric forms of the compounds, the suitable dosages are often in the range of from 0.1 µg to 5000 µg per kilo body weight, such as in the range of from about 0.1 µg to 3000 µg per kilo body weight, and especially in the range of from about 0.1 µg to 1000 µg per kilo body weight. Using multimeric forms of the compounds, the suitable dosages are often in the range of from 0.1 µg to 1000 µg per kilo body weight, such as in the range of from about 0.1 µg to 750 µg per kilo body weight, and especially in the range of from about 0.1 µg to 500 µg per kilo body weight such as in the range of from about 0.1 µg to 250 µg per kilo body weight. In particular when administering nasally smaller dosages are used than when administering by other routes. Administration may be performed once or may be followed by subsequent administrations. The dosage will also depend on the route of administration and will vary with the age and weight of the subject to be treated. A preferred dosage of multimeric forms would be in the interval 1 mg to 70 mg per 70 kg body weight.
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- 35 For some indications a localised or substantially localised application is preferred.

For another application, intranasal application is preferred.

- Some of the compounds of the present invention are sufficiently active, but for some of the others, the effect will be enhanced if the preparation further comprises pharmaceutically acceptable additives and/or carriers. Such additives and carriers will be known in the art. In some cases, it will be advantageous to include a compound, which promote delivery of the active substance to its target.
- In many instances, it will be necessary to administrate the formulation multiple times. Administration may be a continuous infusion, such as intraventricular infusion or administration in more doses such as more times a day, daily, more times a week, weekly, etc. It is preferred that administration of the medicament is initiated before or shortly after the individual has been subjected to the factor(s) that may lead to cell death. Preferably the medicament is administered within 8 hours from the factor onset, such as within 5 hours from the factor onset. Many of the compounds exhibit a long term effect whereby administration of the compounds may be conducted with long intervals, such as 1 week or 2 weeks.
- In connection with the use in nerve guides, the administration may be continuous or in small portions based upon controlled release of the active compound(s). Furthermore, precursors may be used to control the rate of release and/or site of release. Other kinds of implants and well as oral administration may similarly be based upon controlled release and/or the use of precursors.
- Treatment**
- Treatment by the use of the compounds/compositions according to the invention is in one embodiment useful for inducing differentiation, modulating proliferation, stimulate regeneration, neuronal plasticity and survival of cells, for example cells being implanted or transplanted. This is particularly useful when using compounds having a long term effect.
- In further embodiment the treatment may be for stimulation of survival of cells which are at risk of dying due to a variety of factors, such as traumas and injuries; acute

diseases, chronic diseases and/or disorders, in particular degenerative diseases normally leading to cell death, other external factors, such as medical and/or surgical treatments and/or diagnostic methods that may cause formation of free radicals or otherwise have cytotoxic effects, such as X-rays and chemotherapy. In relation to 5 chemotherapy the FGFR binding compounds according to the invention are useful in cancer treatment of all cancer cells presenting FGFRs.

Thus, the treatment comprises treatment and/or prophylaxis of cell death in relation to diseases or conditions of the central and peripheral nervous system, such as 10 postoperative nerve damage, traumatic nerve damage, e.g. resulting from spinal cord injury, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, multiinfarct dementia, multiple sclerosis, nerve degeneration associated with diabetes mellitus, neuro-muscular degeneration, schizophrenia, Alzheimer's disease, Parkinson's disease, or Huntington's disease.

15 Also, in relation to diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as genetic or traumatic atrophic muscle disorders; or for the treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas, such as diabetes mellitus type I and II, of the kidney, such as nephrosis the compounds according to the invention may be used for inducing differentiation, modulating proliferation, stimulate regeneration, neuronal plasticity and survival , i.e. stimulating survival.

25 Furthermore, the compound and/or pharmaceutical composition may be for preventing cell death of heart muscle cells, such as after acute myocardial infarction, in order to induce angiogenesis. Furthermore, in one embodiment the compound and/or pharmaceutical composition is for the stimulation of the survival of heart muscle cells, such as survival after acute myocardial infarction. In another aspect 30 the compound and/or pharmaceutical composition is for revascularisation, such as after injuries.

It is also within the scope of the invention to use the compound and/or pharmaceutical composition for the promotion of wound-healing. The present

compounds are capable of stimulating angiogenesis and thereby promote the wound healing process.

5 The invention further discloses the use of the compound and/or pharmaceutical composition in the treatment of cancer. Regulation of activation of FGFR is important for tumor angiogenesis, proliferation and spreading.

10 In yet a further embodiment the use of the compound and/or pharmaceutical composition is for the stimulation of the ability to learn and/or of the short and/or long term memory, as FGFR activity is important for differentiation of neural cells.

15 In still another embodiment a compound and/or pharmaceutical composition of the invention is for treatment of body damages due to alcohol consumption. Developmental malformations of foetuses, long-term neurobehavioral alterations, alcoholic liver disease is in particular concerned.

Therapeutic treatment of prion diseases including using a compound and/or pharmaceutical composition is still another embodiment of the invention.

20 In particular the compound and/or pharmaceutical composition of the invention may be used in the treatment of clinical conditions, such as Neoplasms such as malignant neoplasms, benign neoplasms, carcinoma in situ and neoplasms of uncertain behavior, diseases of endocrine glands, such as diabetes mellitus, psychoses, such as senile and presenile organic psychotic conditions, alcoholic psychoses, drug psychoses, transient organic psychotic conditions, Alzheimer's disease, cerebral lipidoses, epilepsy, general paresis [syphilis], hepatolenticular degeneration, Huntington's chorea, Jakob-Creutzfeldt disease, multiple sclerosis, Pick's disease of the brain, syphilis, Schizophrenic disorders, affective psychoses, neurotic disorders, personality disorders, including character neurosis, nonpsychotic personality disorder associated with organic brain syndromes, paranoid personality disorder, fanatic personality, paranoid personality (disorder), paranoid traits, sexual deviations and disorders, mental retardation, disease in the nervous system and sense organs, cognitive anomalies, inflammatory disease of the central nervous system, such as meningitis, encephalitis, Cerebral degenerations such as Alzheimer's disease, Pick's disease, 30 senile degeneration of brain, communicating hydrocephalus, obstructive hydro-

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cephalus, Parkinson's disease including other extra pyramidal disease and abnormal movement disorders, spinocerebellar disease, cerebellar ataxia, Marie's, Sanger-Brown, Dyssynergia cerebellaris myoclonica, primary cerebellar degeneration, such as spinal muscular atrophy, familial, juvenile, adult spinal muscular atrophy, motor neuron disease, amyotrophic lateral sclerosis, motor neuron disease, progressive bulbar palsy, pseudobulbar palsy, primary lateral sclerosis, other anterior horn cell diseases, anterior horn cell disease, unspecified, other diseases of spinal cord, syringomyelia and syringobulbia, vascular myopathies, acute infarction of spinal cord (embolic) (nonembolic), arterial thrombosis of spinal cord, edema of spinal cord, subacute necrotic myelopathy, subacute combined degeneration of spinal cord in diseases classified elsewhere, myelopathy, drug-induced, radiation-induced myelitis, disorders of the autonomic nervous system, disorders of peripheral autonomic, sympathetic, parasympathetic, or vegetative system, familial dysautonomia [Riley-Day syndrome], idiopathic peripheral autonomic neuropathy, carotid sinus syncope or syndrome, cervical sympathetic dystrophy or paralysis, peripheral autonomic neuropathy in disorders classified elsewhere, amyloidosis, diseases of the peripheral nerve system, brachial plexus lesions, cervical rib syndrome, costoclavicular syndrome, scalenus anterior syndrome, thoracic outlet syndrome, brachial neuritis or radiculitis, including in newborn. Inflammatory and toxic neuropathy, including acute infective polyneuritis, Guillain-Barre syndrome, Postinfectious polyneuritis, polyneuropathy in collagen vascular disease, disorders affecting multiple structures of eye, purulent endophthalmitis, diseases of the ear and mastoid process, chronic rheumatic heart disease, ischaemic heart disease, arrhythmia, diseases in the pulmonary system, abnormality of organs and soft tissues in newborn, including in the nerve system, complications of the administration of anesthetic or other sedation in labor and delivery, diseases in the skin including infection, insufficient circulation problem, injuries, including after surgery, crushing injury, burns. Injuries to nerves and spinal cord, including division of nerve, lesion in continuity (with or without open wound), traumatic neuroma (with or without open wound), traumatic transient paralysis (with or without open wound), accidental puncture or laceration during medical procedure, injury to optic nerve and pathways, optic nerve injury, second cranial nerve, injury to optic chiasm, injury to optic pathways, injury to visual cortex, unspecified blindness, injury to other cranial nerve(s), injury to other and unspecified nerves. Poisoning by drugs, medicinal and biological substances, genetic or traumatic atrophic muscle disorders; or for the treatment of diseases or con-

ditions of various organs, such as degenerative conditions of the gonads, of the pancreas, such as diabetes mellitus type I and II, of the kidney, such as nephrosis. Scrapie, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Sheinker (GSS) disease.

5

According to invention the treatment and/or prevention of the above conditions and symptoms comprises a step of administering an effective amount of an compound and/or pharmaceutical composition of the invention to an individual in need.

10

Examples

Example 1. Synthesis of FGL

Solid Phase Synthesis

15

The individual peptide chain of FGL was synthesized by the standard Fmoc-solid phase method (see for example WO 98/11125). The synthesis was performed on TentaGel S RAM resin (90 mg, 0.22 mmol/g). The resin was placed in a polyethylene vessel equipped with a polypropylene filter for filtration. The resin was swelled in DMF, and treated with 20% piperidine in DMF to secure the presence of non-protected amino groups on the resin. Afterwards the resin was drained and washed with DMF until no yellow colour could be detected after addition of Dhbt-OH to the drained DMF. The amino acids were coupled one at a time alternating with removal of Fmoc-groups. The amino acid derivatives used for the synthesis of FGL were, (in the following order):

Fmoc-Ala-OH

Fmoc-Lys(Boc)-OH

Fmoc-Ser(tBu)-OH

Fmoc-Lys(Boc)-OH

30

Fmoc-Gly-OH

Fmoc-Gln(Trt)-OH

Fmoc-Gln(Trt)-OH

Fmoc-Asn(Trt)-OH

Fmoc-Glu(tBu)-OH

35

Fmoc-Ala-OH

Fmoc-Val-OH

Fmoc-Val-OH
Fmoc-Tyr(tBu)-OH
Fmoc-Val-OH
Fmoc-Glu(tBu)-OH

5

The Fmoc-amino acids were preactivated in DMF by TBTU /HOBr before the coupling to the growing resin-bound peptide chain. For the removal of Fmoc-groups piperidine in DMF was used. At the end of the solid phase synthesis, the peptide chain bound to the resin looked as the follows:

10

Glu(tBu)-Val-Tyr(tBu)-Val-Val-Ala-Glu(tBu)-Asn(Trt)-Gln(Trt)-Gln(Trt)-Gly-Lys(Boc)-Lys(Boc)-Ser(tBu)-Lys(Boc)-Ala-R

Dimerisation

15

The dimer was created by coupling Boc-iminodiacetic acid to the peptide on the resin, using TBTU/HOBt as described in WO 00/18791. To reduce side reactions multiple coupling were performed with Boc-iminodiacetic acid as the limiting component.

20

Cleavage and purification

25

The peptide was cleaved from the resin and simultaneously deprotected on the side chains in TFA at the presence of TES and water as scavengers to yield the peptide amide. The amount of TFA was reduced by evaporation and the peptide precipitated.

Final purification of FGL₄ was done by reversed phase HPLC. The conditions of HPLC were as follows:

Column: YMC ODS-AMQ, 5 µm, 4.6 x 250 mm, 200 A

30

Flow: 1.0 ml/min

Mobile phases: A: 0.12% TFA, 95% H₂O, 5% ACN

B: 0.1% TFA, 95% ACN, 5% H₂O

Gradient: 15% B to 35% B in 20 min,

35% B to 95% B in 1 min and hold for 5 min.

35

Detection: Diode array detector 190-400 nm

Wavelength: 220 nm
Injection volume: 20 µl,
Concentration of FGL_L 0.5 mg/ml

5 The HPLC purified FGL_L was isolated by lyophilisation.

The flowchart of FGL_L synthesis is shown in Figure 1. Figure 2 depicts the HPLC elution profile of the final purification of FGL_L.

Starting materials, reagents and solvents used in the production of FGLL

10 Acetic acid, Acetic acid anhydride, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, Boc-iminodiacetic acid, t-Butyl methyl ether, Dimethylformamide, Ethanol, 99,9%, N-Ethyl-diisopropylamine, Fmoc-Ala-OH, mocl-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH, 1-
15 Hydroxybenzotriazol, Isopropanol, N-methylpyrrolidone, 1-Octanol, Piperidine, Trifluoroacetic acid, Triethylsilane

Abbreviations

Abbreviations for amino acids are in accordance with the recommendations in the
20 IUPAC-IUB Joint Commission on Biochemical Nomenclature Eur. J. Biochem., 1984,
vol. 184, pp 9-37

Other abbreviations:

AcOH Acetic acid
25 TBTU O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
Ida Boc iminodiacetic acid
MTBE t-Butyl methyl ether
DMF Dimethylformamide
EtOH Ethanol, 99,9%
30 DIPEA N-Ethyl-diisopropylamine
HOBr 1-Hydroxybenzotriazol
NMP N-methylpyrrolidone
TFA Trifluoroacetic acid
TES Triethylsilane
35 Boc N-tertButyl oxycarbonyl

Fmoc 9-Fluorenylmethyloxycarbonyl

tBu tert-Butyl

HPLC High pressure liquid chromatography

5 R Amide-TG-resin

AA Amino acid

Physico-chemical properties of FGL_L

FGL_L has the molecular formula C₁₄₆H₂₃₇N₄₃O₅₀ · (CH₃CO₂H)_x · (H₂O)_y and the structural formula:



HN\



15

It consists of two identical peptide sequences of 15 amino acid residues co-joined by the linker group, amidoacetic acid (N-(carboxymethyl)glycine), on their N-terminals. The molecular weight of FGL_L is 3394.8 Dalton. In water solutions at concentrations of 0.5 mg/ml and higher, FGL_L tends to non-covalent aggregation. Aggregation is prevented by addition of acetic acid.

25

The time course of aggregation was studied for a wide range of concentrations (0.5 – 25 mg/ml) of FGL_L using pharmaceutical formulations suitable for intranasal administration in clinical trials, i.e. isotonic glucose containing parabenoate (1.5 % parabenoate, 5.5 % glucose in water). Aggregation was evaluated by analytical HPLC after 5, 10, 15, 30, and 60 minutes, and also after 24 hours. The results of HPLC showed that the formation of aggregates of FGL_L is concentration and time dependent. After 24 hours, only 13.5% of the FGL_L was found in aggregates when concentration of the compound was 0.5 mg/ml. At concentration 25 mg/ml aggregation was more rapid, with approximately 40% conversion of soluble FGL_L to aggregates. However, the later concentration of FGL_L (25 mg/ml) was successfully used in vivo trials for intranasal administration in the dog with no evidence of local or systemic effects indicating that in physiological conditions the aggregates of FGL_L are reversed to solution.

30
35

Example 2. Synthesis of FGL_d

The FGL_d is synthesised according the method described in PCT/US90/02039.

Figure 3 demonstrates the HPLC profile of the final purification of FGL_d. The

5 hatched area indicates the level of heterogeneity of the synthesised product.

**Example 3. The effect of different formulations of FGL on survival of neurons
in vitro**

10 To study whether the FGL peptide possesses a stimulatory effect on neuronal cell survival in vitro three types of neuronal cell primary culture were used :

Dopaminergic neurons (DN)

15 Dopaminergic neurons were prepared from Wistar rat embryos at embryonic day 15 (Charles River, Sulzfeld, Germany or Møllegaard, Denmark). A pregnant rat was sacrificed and the uterus was taken out and kept on ice in Hank's balanced salt solution (HBSS; Gibco, BRL). The ventral part of the mesencephalon was dissected from the embryonic brains, homogenised on ice in Gey's balanced salt solution (GBSS; Gibco, BRL) supplemented with 5 g glucose/l (Sigma-Aldrich) and thereafter tryp-
20 sinised. The dissociated cells were washed in the presence of DNase 1 and soy-bean trypsin inhibitor (Sigma-Aldrich).

For the survival assay isolated neurons were seeded at a density of 150,000 cells/cm² in 24-well cell culture plates coated with poly-D-lysine as described above. The neurons were left to differentiate for six days without or with various concentrations of the FGL peptide, after which 6-OHDA was added at a concentration of 100 µM for two hours. A stock solution was prepared by dissolving 6-OHDA to a concentration of 10 mM in 0.1 % (w/v) sodium metabisulfite in order to prevent oxidation. After two hours with 6-OHDA, the medium was changed to Neurobasal medium with supplements and peptide, and the cell cultures were further incubated for 24 hours, 30 fixed and immunostained for tyrosine hydroxylase. 98 images for each experimental condition in each individual experiment were automatically recorded as described for the dopaminergic neurite outgrowth assay below.

Hippocampal neurons (HN)

Dissociated hippocampal neurons were isolated from Wistar rat embryos at embryonic day 19 or newborn rats as previously described by Rønn et al. (1999). Briefly, hippocampus was isolated from the brain in ice cold modified Krebs Ringer solution, cleared of blood vessels, roughly homogenised by chopping and then trypsinised.

- 5 The dissociated cells were washed in the presence of DNase 1 and soybean trypsin inhibitor.

For the assay neurons were plated at a density of 40,000 cells/cm² on 8-well permanox slides coated with 10 µg/ml poly-L-lysine in Neurobasal medium. 15 minutes after plating, Aβ 25-35 was added to a final concentration of 20 µM. The Aβ 25-35 had prior to plating been dissolved in sterile deionized water to a concentration of 3 mM and incubated at 37°C for four days (Pigino et al., 2001; Grace et al., 2002). This treatment has previously been shown to induce fibrillation of the Aβ-peptide fragment, thereby increasing its neurotoxic activity (Lorenzo et al., 1994). The neurons were incubated with Neurobasal medium containing the FGL peptide for 70 hours, 10 fixed with 4 % (v/v) formaldehyde, and stained with Hoechst 33258 for 25 minutes as described by Kruman et al. (1997). Images of 1000-1500 neurons were randomly recorded for each group in each experiment using computer assisted fluorescence microscopy as described for the hippocampal neurite outgrowth assay. Nuclei from dead and live neurons were counted using the software package Prima developed at 15 the Protein Laborator, Copenhagen University, and the fraction of live neurons relative to the total number of neurons was estimated.

- 20
- Cerebellar granule neurons*
- 25 Cerebellar granule neurons (CGN) were prepared from postnatal day seven Wistar rats largely as previously described by Schousboe et al. (1989). Cerebellar tissue was dissected in modified Krebs-Ringer solution kept on ice, and treated as described for the hippocampal neurons above. All cell cultures were incubated at 37°C in a humidified atmosphere containing 5 % CO₂. All animals were handled in accordance with the national guidelines for animal welfare.
- 30 Primary cultures of CGN were plated at a density of 100,000 cells/cm² on poly-L-lysine coated 8-well permanox slides in Neurobasal-A medium (Gibco, BRL) supplemented with 2 % (v/v) B27, 0.5 % (v/v) glutamax, 100 U/ml penicillin, 100 µg/ml streptomycin and KCl, making the final concentration of KCl in the medium 40 mM. 24 hours after plating, cytosine-β-D-arabinofuranoside (Ara-C; Sigma-Aldrich) was

added to a final concentration of 10 µM to avoid proliferation of glial cells, after which the neurons were allowed to differentiate for further six days at 37°C. Apoptotic cell death was induced by washing twice and changing the medium to Basal Medium Eagle (BME; Gibco BRL) supplemented with 1 % (v/v) glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, 3.5 g D-glucose/l and 1 % (v/v) sodium pyruvate (Gibco BRL) together with various concentrations of peptide. Thereby the concentration of potassium in the cultures was reduced to 5 mM KCl (D'Mello et al., 1993). Two days after induction of apoptosis, the cells were fixed with 4 % formaldehyde and stained with Hoechst 33258 as described for the survival assay employing hippocampal neurons.

Other survival assays using the cultures of CGN.

1. PACE assay

Detection of phosphorylated Erk1/2 and Akt relative to the total number of CGN was carried out largely according to Versteeg et al. (2000). CGN were plated at a density of 290,000 cells/cm² in poly-L-lysine coated 96-well microtiter plates. The neurons were grown in Neurobasal-A medium supplemented with 0.5 % (v/v) glutamax, 100 U/ml penicillin and 100 µg/ml streptomycin. 24 hours after plating, Ara-C was added to the cultures as described above. After 72 hours of incubation, half of the medium was replaced with Neurobasal-A medium containing the peptide to be tested. For the Erk1/2 phosphorylation assay, the cultures were further incubated for 0 - 90 minutes, centrifuged at 70 g for 8 minutes, fixed with 4 % (v/v) formaldehyde and immunostained using primary phospho-p42-44 rabbit antibodies and peroxidase-conjugated anti-rabbit secondary antibodies. For the detection of phosphorylated Akt, the cultures were further incubated for 10 or 30 minutes, centrifuged at 70 g for 8 minutes, fixed with 4 % (v/v) formaldehyde and immunostained using polyclonal antibodies against Akt phosphorylated at Ser473 and peroxidase-conjugated secondary antibodies. The total number of neurons was in both phosphorylation-assays estimated by staining with crystal violet.

30

2. TUNEL assay

CGN were plated at a density of 60,000 cells/cm² on poly-L-lysine coated 8-well permanox slides. The cultures were grown in Neurobasal-A medium supplemented with 2 % (v/v) B27, 0.5 % (v/v) glutamax, 100 U/ml penicillin, 100 µg/ml streptomycin

and 40 mM KCl (final concentration) for six days, and apoptosis was induced as described for the CGN survival assay above. 24 hours after induction of apoptotic cell death, the cultures were fixed and the extent of DNA-fragmentation was measured using the ApoAlert apoptosis detection kit, essentially as described by the manufacturer. Briefly, blunt ends of double-stranded DNA molecules were enzymatically labelled with fluorescein-dUTP, and all the neurons were stained with propidium iodide at a concentration of 750 ng/ml. Images of at least 200 neurons from each group in each experiment were obtained using a BioRad Radiance laser scanning system 2000 coupled to a Nikon Eclipse TE 200 confocal microscope equipped with an oil immersion 60x 1.4 NA objective (Nikon, Tokyo, Japan). The fraction of TUNEL-positive neurons was determined by counting.

RESULTS

15 *FGL promotes survival of dopaminergic, hippocampal and cerebellar granule neurons*

Since FGL is an agonist of the FGF receptor with a growth factor-like activity, it was investigated whether FGL could act as a neuroprotectant under various neurotoxic conditions. Cell death in dopaminergic neurons was induced by addition of 6-OHDA. In Figure 4a it can be seen that the number of live dopaminergic neurons was strongly decreased after exposure to 6-OHDA as compared to the untreated control. Addition of 10 ng/ml glia derived neurotrophic factor (GDNF) partially prevented this neuronal loss (150 % as compared to the 6-OHDA-treated control cells set to 100 %). When cultures were grown in the presence of various concentrations of FGLd (Figure 4b), survival of dopaminergic neurons treated with 6-OHDA increased statistically significantly with a maximal rescue of approximately 135 % at a concentration of 1 µg/ml FGLd and the effect exhibited a bell-shaped dose-response relationship.

30 Thus, FGLd is able to rescue dopaminergic neurons to approximately the same extent as GDNF in a survival model employing 6-OHDA as the neurotoxic compound.

It was also tested whether FGLd is able to protect hippocampal neurons from the death induced by Aβ 25-35. Aβ 25-35 has previously been shown to be neurotoxic in primary cultures of foetal hippocampal neurons (Piké et al., 1995; Køhler et al., 2003). Cultures of hippocampal neurons were incubated with pre-aggregated Aβ 25-

35 together with various concentrations of FGLd. In Figure 4c it can be seen that treatment of hippocampal cell cultures with 20 µM A β 25-35 induced a moderate-low neuronal cell loss. This loss could in part be rescued by 50 ng/ml brain derived neurotrophic factor (BDNF). As shown in Figure 4d, treatment with FGLd in concentrations ranging from 0.1 – 50 µg/ml also partly rescued hippocampal neurons from neurodegeneration induced by 20 µM A β 25-35. The FGLd-induced neuroprotection reached a maximal level already at a concentration of 0.3 µg/ml FGLd (110 % as compared to the A β 25-35-treated controls), remaining more or less constant at concentrations of FGLd up to 50 µg/ml.

10

Thus, FGLd can protect hippocampal neurons against the neurotoxic effect of the A β 25-35 peptide to the same extent as that achieved by treatment with BDNF.

15

It has previously shown that if primary cultures of CGN are grown at high levels of KCl, and the concentration of KCl subsequently is reduced, apoptosis is induced (D'Mello et al., 1993; Pedersen et al., 2004). A neuroprotective effect of FGLd was investigated in CGN cultures induced to differentiate in 40 mM KCl for seven days. Subsequently the KCl concentration was reduced to 5 mM, and after two days, cell viability was estimated. As seen in Figure 4e, a low KCl concentration induced cell death in CGN cultures, and this could be rescued by treatment with insulin-like growth factor 1 (IGF-1). As shown in Figure 4f, cell death could also be partially prevented by treatment with FGLd. A maximal neuroprotection of 135 % as compared to controls grown at a low KCl concentration was seen at peptide concentrations between 1 - 10 µg/ml.

25

Thus, the results indicate that FGL is capable of promoting survival of dopaminergic, hippocampal and cerebellar granule neurons in vitro.

FGL protects cerebellar granule neurons against apoptosis

30

In order further to characterise the mechanism of the neuroprotective effects of FGLd, the extent of DNA-fragmentation (normally associated with apoptosis) in KCl-deprived CGN was measured (Eldadah et al., 2000; Gerhardt et al., 2001). CGN maintained in the high KCl medium displayed very few neurons with fragmented DNA. A large proportion of the cells treated with low KCl displayed DNA-fragmentation. In CGN cultures treated with FGLd in a concentration of 10-20 µg/ml,

very few of the neurons displayed fragmented DNA upon KCl-deprivation. In contrast, the control peptide, FGL9, 10diAlad, with a double alanine substitution exhibited no neuroprotective effect. The neuroprotective effect of FGLd was subsequently quantified, and in Figure 5 it can be seen that KCl-deprivation resulted in a clear
5 increase of the number of CGN containing fragmented DNA as compared to CGN maintained in a high KCl medium. Treatment with the control peptide, FGL9, 10diAlad, did not rescue CGN from apoptosis. Addition of FGLd in various concentrations reduced the number of apoptotic CGN with a maximal rescue of 40 % at concentrations of 10 – 15 µg/ml FGLd, relative to apoptotic neurons without FGLd-
10 treatment.

FGL-induced signal transduction results in phosphorylation of Erk and Akt

The FGL peptide has been shown to bind and activate the FGFR (Kiselyov et al., 2003). Signalling pathways initiated by the FGFR include among others the Ras-
15 MAPK pathway, as reflected by activation of MAPK Erk1/2, and the PI3K pathway, which activates the serine/threonine protein kinase Akt. Both pathways are known to be involved in neuronal differentiation and survival. The neurite outgrowth and survival promoting effects of FGL may therefore depend on activation of the MAPK and PI3K pathways. It was therefore investigated whether Erk1/2 and Akt were phospho-
20 rylated in response to treatment of CGN cultures with the FGL peptide. From Figure 8 it appears that the peptide in a concentration of 10 µg/ml induced a sustained phosphorylation of Erk1/2 in contrast to a concentration of 5 µg/ml, which was unable to induce phosphorylation of Erk1/2. This activation (115-120 %, as compared to control cultures) was seen 10 minutes after stimulation with FGLd and lasted for at
25 least 90 minutes.

The phosphorylation of Akt by FGL was analysed after exposure of CGN cultures to the peptide for 10 or 30 minutes (Figure 7). A 10-minute exposure period in concentrations above 0.5 µg/ml led to a significant increase of phosphorylation of Akt as compared to the untreated control. The phosphorylation level reached a maximum at
30 a peptide concentration of 1 µg/ml, and stayed relatively constant at doses increasing up to 20 µg/ml. At exposure times of 30 minutes, no phosphorylation of Akt was detected indicating that activation of Akt was transient and terminated within 30 minutes in contrast to the phosphorylation response of Erk1/2.

Thus, the results indicate that FGL induces a sustained (at least 90 minutes) activation of the MAPK pathway and a transient (10 minutes) activation of Akt.

FGL promotes neuronal cell survival through FGFR, MEK and PI3K

5 In order to investigate whether the FGFR, MEK and PI3K also were involved in FGL-induced survival, CGN cultures induced to undergo apoptosis were treated with the above-described inhibitors. As shown in Figure 8, survival of cells in the apoptosis-induced CGN cultures treated with FGLd, but without inhibitors, were set to 100 %, and the apoptosis-induced control neurons not treated with FGLd (marked with a dashed line) had a lower survival rate, as compared to the FGLd-treated neurons.
10 The FGFR inhibitor, SU5402 (■), significantly inhibited the survival response to FGLd already at a concentration of 20 μ M and a concentration of 40 μ M SU5402 reduced the FGLd-induced survival to the survival rate of the untreated apoptosis-induced control neurons. The MEK inhibitor, PD98059 (●), inhibited cell survival induced by
15 FGLd to the level of the control cultures at a concentration of 12.5 μ M. The PI3K inhibitor LY294002 (▲) inhibited FGLd-induced cell survival significantly at a concentration of 3.5 μ M, indicating that LY294002 is a very potent and general inhibitor of neuronal survival.

20 Thus, the FGL-induced survival after KCl-deprivation of CGN cultures is dependent on activation of the FGFR and of the intracellular kinases MEK and PI3K. The results show that LY294002 is the most potent inhibitor of neuronal survival, followed by the MEK inhibitor, PD98059, which appeared to be less efficient in the neurite outgrowth assay than in the survival assay (see below), possibly indicating that the main functions of the FGL-induced MEK activation is neuroprotection, rather than differentiation.
25

Example 4. *In vitro* neurite outgrowth stimulation by different formulations of FGL

Primary cultures of DN, HN and CGN were prepared as described above.

30 **Dopaminergic neurons (DN)**

DN were plated at a density of 100,000 cells/cm² in 24-well cell culture plates (previously coated with 12.5 μ g/ml poly-D-lysine; Sigma-Aldrich) in a medium containing 50 % (v/v) Optimem 1 (Gibco, BRL), 25 % (v/v) horse serum (Gibco, BRL), 25 % (v/v) HBSS and 5 g glucose/l. The cells were left to adhere for one-two hours, before

the medium was changed to Neurobasal medium supplemented with 2 % (v/v) B27 Neurobasal Supplement, 0.5 % (v/v) glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (all from Gibco, BRL) without or with various concentrations of peptide. After 72 hours of incubation, the neurons were fixed with 4 % (v/v) formaldehyde and immunostained using a primary mouse monoclonal antibody against tyrosine hydroxylase and secondary biotinylated sheep anti-mouse antibodies followed by incubation with peroxidase conjugated streptavidin. 98 images for each experimental condition in each individual experiment were automatically recorded using a computerised microscope workstation described by Walmod et al. (2002). Briefly, the workstation consisted of an Eclipse TE 300 inverted microscope (Nikon, Tokyo, Japan) equipped with a motorised, movable microscope stage (LUDL Electronic Production Ltd, Hawthorne, NY, USA), a 10x objective and a 1100 analogue b/w CCD video camera (DFA, Denmark). A software package Prima developed at the Protein Laboratory (Copenhagen University, Denmark) was used to make a stereologically based determination of neurite length (Rønn et al., 2000).

Hippocampal neurons (HN) and cerebellar granule neurons (CGN)

Postnatal HN or CGN were plated at a density of 10,000 cells/cm² on uncoated 8-well permanox Lab-Tek chamber slides in Neurobasal medium supplemented with 0.4 % (w/v) bovine serum albumin (BSA; Sigma-Aldrich), 2 % (v/v) B27 Neurobasal supplement, 1 % (v/v) glutamax, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 % 1 M HEPES (all from Gibco, BRL). Peptide solutions without or with inhibitors of various signal transduction pathways were added to a total volume of 300 µl/cm², and the slides were incubated at 37°C. After 24 hours, the neurons were fixed with 4 % (v/v) formaldehyde for 20 minutes and thereafter immunostained using primary rabbit antibodies against GAP-43 and Alexa Fluor secondary goat anti-rabbit antibodies. Images of at least 200 neurons for each group in each individual experiment were obtained systematically by using computer assisted fluorescence microscopy as previously described (Rønn et al., 2000). Briefly, a Nikon Diaphot inverted microscope with a Nikon Plan 20x objective (Nikon, Tokyo, Japan) coupled to a video camera (Grundig Electronics, Germany) was used for recordings. The same software package as described above for the dopaminergic neurite outgrowth assay was used to process the recorded images.

Results

FGL induces neurite outgrowth in primary cultures of dopaminergic, hippocampal and cerebellar granule neurons

A monomeric form of FGL has recently been demonstrated to induce neurite outgrowth from primary hippocampal neurons (Kiselyov et al., 2003). It is known that multimeric forms of peptide ligands have a higher potency for receptor activation than monomeric forms (Rønn et al., 1999). Therefore, various dimeric and tetrameric forms of FGL were manufactured and tested on their neurite outgrowth stimulatory capability employing primary cultures of dopaminergic, hippocampal and cerebellar granule neurons (CGN).

The cultures were grown in the absence or presence of the tetrameric (dendrimeric) FGL peptide (FGLd). Neurite outgrowth induced by FGLd in all three types of neurons was subsequently quantified and shown in Figure 9. Dopaminergic neurons treated with FGLd (●) exhibited a statistically significant increase in neurite length in a dose-dependent manner with a maximal average neurite length (125 % as compared to the untreated control) obtained at a concentration of 1 µg/ml FGLd. Hippocampal neurons treated with FGLd (▲) exhibited an increase in neurite length already at a dose of 0.04 µg/ml FGLd, reaching a plateau-like level with a stimulation of approximately 150 % as compared to the untreated control cultures employing concentrations of FGLd up to 20 µg/ml. CGN cultures treated with FGLd (■) also exhibited a dose-dependent neurite outgrowth response. A maximal stimulation of 255 % as compared to the untreated control was seen at a concentration of 50 µg/ml FGLd.

Primary cultures of hippocampal neurons were selected to test the effect of three different dimeric versions of the FGL peptide, FGL_L, FGL_{lys} and FGL_{cys}, on neurite outgrowth. As shown in Figure 10, the dimeric lysine dendrimers, FGL_{lys} (▲) and FGL_{cys} (●) were both unable to stimulate neurite outgrowth at any of the concentrations tested. In contrast, FGL_L (■) induced a significant neurite outgrowth response exhibiting a dose-dependent relation with a bell-shaped curve reaching a maximal stimulation at a concentration of 1 µg/ml. The maximal stimulation was 155 % as compared to the untreated control (Figure 10), which is roughly the same as the response induced by FGLd (see Figure 9).

Neurite outgrowth induced by FGL involves activation of FGFR, MEK and PI3K

In order to elucidate the biological correlates of activation of FGFR, MEK and PI3K, it was tested the effect of inhibitors of these kinases on neurite outgrowth induced by FGLd in CGN. The FGFR inhibitor, SU5402, is known to inhibit the tyrosine kinase activity of the FGFR subtype 1 by interacting with the catalytic domain of the receptor. In Figure 11 the average neurite length in FGLd-treated CGN cultures is set at 100 % and the average neurite length for control neurons not treated with FGLd is marked with a dashed line. It appears that SU5402 (■) significantly inhibited the FGLd-induced neurite outgrowth at a concentration of 80 µM (to 35 % of the initial value). Treatment with the MEK inhibitor PD98059 (●) in concentrations above 25 µM statistically significantly decreased neurite outgrowth induced by FGLd to 75 %. Also, the PI3K inhibitor LY294002 (▲) significantly inhibited FGLd-induced neurite outgrowth already at a concentration of 3.5 µM to 90 % and at a concentration of 10 µM LY294002 the neurite outgrowth response to FGLd was reduced to almost 50 %, which is lower than for the untreated control neurons. SU5402 and LY294002 were without significant effect on neurite outgrowth in the control cultures not treated with FGLd (data not shown). PD98059 has previously been found not to affect the basal neurite outgrowth in CGN (Drosopoulos et al., 1999). Thus, the FGLd-induced neurite outgrowth response of CGN is dependent on the activation of the FGFR and the subsequent activation of the MAPK and the PI3K intracellular signalling pathways.

20

Table 1. Summary of the effects of different formulations of the FGL peptide on neuronal survival in primary cultures of rat CGN, DN and HN induced to undergo cell death by withdrawal of high potassium, or addition of 6-hydroxy dopamine (6-OHDA) or (25-35) β-amylloid peptide fragment .

25

Mechanism for inducing cell death	Tissue source (age of rat)	FGL peptide/ effective concentration	Neuronal Survival (% of Control) ^a
Low K ⁺ (5 mM)	CGN (PND 3-4)	<i>FGL_m</i> : 250 µg/mL	125%*
Low K ⁺ (5 mM)	CGN (PND 3-4)	<i>FGL_d</i> : 5 µg/mL 20 µg/mL	110%* 117%**
Low K ⁺ (5 mM)	CGN (PND 3-4)	<i>FGL_d</i> : 1 µg/mL 10 µg/mL	131%* 132%*

Low K ⁺ (5 mM)	CGN (PND 7-8)	FGL _m : 10 µg/mL 50 µg/mL 250 µg/mL	110%* 119%* 142%*
Low K ⁺ (5 mM)	CGN (PND 7-8)	FGL _d : 20 µg/mL	175%*
6-OHDA	DA (E14)	FGL _d : 1 µg/mL	143%*
β-amyloid	Hippocampal (E19)	FGL _d : 0.01 µg/mL 3 µg/mL 9 µg/mL	109%** 107%** 107%**
β-amyloid	Hippocampal (E19)	FGL _L : 0.3 µg/mL	108%*

^a Survival in cultures induced to undergo cell death without FGL treatment is designated as 100% (control) and compared to cultures where FGL was added.

Statistics (+FGL vs. control treatment): *p<0.05; **p<0.01, ***p<0.001

5

Table 2. Summary of the effects of FGL peptides on neurite outgrowth in primary cultures of rat CGN, DN and HN.

Statistics (+FGL vs. Control): *p<0.05; **p<0.01; ***p<0.001

10 PND = Post-natal day; E = Embryonic day

Assay	Tissue Source/ Age of Rat	Peptide Concentra- tion (µg/mL)	Effect on Neurite Length (Percent Increase vs. Con- trol)
Primary culture; Incubation: 24 h +/- FGL; Analysis: computer as- sisted fluorescence mi- croscopy	CGN/ - PND 2-4 - PND 5-8	FGL _d - 9 FGL _d - 3, 9, 27 FGL _d - 10, 25, 50, 100	24%** 30%**, 93%**, 121%* 37%*, 79%*, 179%**, 96%**
Primary culture; Incubation: 72 h +/- FGL; Analysis: computer as- sisted fluorescence mi- croscopy	DN/ E14	FGL _d - 0.04, 0.2, 1, 5 FGL _L - 0.3, 1, 3 FGL _m - 10, 50, 250	12%***, 23%***, 24%***, 13%* 19%*, 34%**, 33%** 15%*, 23%***, 28%***
Primary culture; Incubation: 24 h +/- FGL; Analysis: computer as- sisted fluorescence mi- croscopy	HN/ - E19 - Newborn	FGL _d - 3, 9, 27 FGL _d - 0.04, 5 FGL _L - 0.2, 1, 5	65%*, 51%*, 69%* 38%**, 45%* 35%**, 53%**, 30%**

croscopy			
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Conclusions

- 5 1. The FGL peptide is a survival promoting compound for a variety of neuronal cell types in vitro, which is essential in the treatment of neuro-degenerative disorders.
- 10 2. The FGL peptide is capable of inducing neurite outgrowth in a variety of neuronal cell types in vitro and FGL has a potential to promote the growth of both axons and dendrites, which is essential in the treatment of neuro-degenerative disorders.
- 15 3. All, monomeric FGL_m, dimeric FGL_L and dendrimeric FGL_D, formulations of the FGL peptide are potent in promotion of both survival and neurite outgrowth.
4. FGL_L and FGL_D both are more than 200 times more potent than FGL_m in promotion of neurite outgrowth.
5. FGL_L is as potent in promotion of both neurite outgrowth and survival as FGL_D.
6. Other formulations of the FGL peptide such as dimers FGL_{lys} and FGL_{cys} are inactive in promotion of neurite outgrowth.

20 **Example 5. The effect of FGL on synaptic plasticity of primary cell cultures of rat hippocampal neurons**

In the adult nervous system, structures such as the hippocampus continuously undergo plastic changes to adjust structure and function. In particular, the acquisition of memory is believed to require structural and functional changes of already established neuronal connections. NCAM has been shown to play an important role in neuronal plasticity during development and in learning and memory [Welzl and Stork, 2003]; however, the mechanism for these effects is unknown. The present study it was investigated whether the FGL peptide is able to affect synaptic plasticity.

The effects of FGL_d on vesicle turnover was estimated in primary cultures of hippocampal neurons from embryonic rats (E19). The cultures were prepared as above. Following 13-16 days in vitro, FGL_d was added to the cultures and incubated for 1,

24 or 48 hours. After 48 hours of incubation, the FGFR-inhibitor (SU 5402) was added simultaneously with FGL_d to some of the cultures.

After treatment with FGL_d the neurons were labeled with the fluorescent membrane probe FM 1-43 as described by Ryan et al. [1993] and Kiryushko et al [2003]. The rate of FM 1-43 release (destaining of cells) evoked by depolarisation with 90 mM potassium was then evaluated by a time-lapse image acquisition using the Radiance 2000 scanning system and a Nikon eclipse TE200 confocal microscopy.

The effect of FGL_d on synapse formation was further evaluated using double-immunostaining of neurites and synapses in primary embryonic (E19) hippocampal neurons grown in culture for 13 days. Cells were incubated with FGL_d for 48 or 96 hours, before-fixation and immunostaining as described by Kiryushko et al [2003]. The synapses were detected by antibodies against synaptophysin and the neurites were labelled by antibodies against the growth-associated protein-43 (GAP-43- also called B-50 or F1). The immunostaining was visualised and quantified by confocal fluorescence microscopy as described by Rønn et al. [2000].

Results

The treatment of hippocampal neurons with 20 µg/ml FGL_d for 1 h and 48 h resulted in a statistically significant increase of the rate of FM 1-43 destaining, compared with the control (from 0.008 sec⁻¹ to 0.016 sec⁻¹, and from 0.013 sec⁻¹ to 0.028 sec⁻¹, respectively); whereas 24 h treatment did not result in a significant effect (Figure 12). This suggests that FGL_d causes both a short-term facilitation of transmitter release (1 h) and a long-term (48 h) increase of synaptic efficiency. The FGFR inhibitor SU5402 abolished the effect of FGL_d indicating that the effect of FGL_d was mediated through its interactions with FGFR.

The double immunostaining of hippocampal neurons for synaptophysin and GAP-43 showed that FGL_d (20 µg/ml) administered to hippocampal cultures for 48 and 96 hours significantly increased the number of synapses, as reflected by approximately 20% increase in the number of synaptophysin positive spots per 100 µm length of neurite (from 2.40 to 2.96 and from 2.73 to 3.24, respectively).

These results suggest that the FGL peptide influences synaptic plasticity by increasing the rate of presynaptic vesicle turnover and the number of the synapses.

Example 6. The effect of FGL on organotypic hippocampal neuronal slice cultures and primary cultures of dissociated hippocampal neurons exposed to ischemic conditions

5

The brain has a high consumption of oxygen and glucose and depends almost exclusively on oxidative phosphorylation for energy production [Dirnagl et al, 1999].

Deficiency of oxygen and glucose supply results in neuronal cell damage and death [Lipton, 1999; Lee et al, 2000]. The susceptibility to ischemic injury differs among

10 different parts of the brain and even different neuronal populations. The neurons of the hippocampal CA1 area are one of the regions in the brain of high vulnerability [Gao et al, 1998; Krebs et al, 2003].

Methods

15 In the present study it was investigated the effect of the FGL peptide on synaptic
plasticity, neuronal function and vulnerability following oxygen and glucose depriva-
tion (OGD) in organotypic hippocampal neuronal slice cultures (OHSC) from 7-day-
old Wistar rats [method of Stoppini et al, 1991] and primary cultures of dissociated
hippocampal neurons from 1-day-old rats [method of Maar et al, 1997]. Primary cell
cultures were maintained for 11-12 days and OHSC for 12-14 days prior to adding
FGL_d.

The primary cell cultures and OHSC were exposed to OGD for 10 and 20 minutes. Following OGD, the cultures were again supplied with oxygen and glucose and analysed at 1, 4 or 24 hour time point after introduction of OGD exposure. FGL_d (or the control peptide FGL_c) was added 24 hr before, immediately, or 1 hr or 24 hr after OGD. When administered prior to OGD, FGL_d was removed from the medium when introducing OGD. In some cases, the slice cultures were treated with FGL_d only at 4 hr after OGD. In addition, the FGFR inhibitor, SU5402 (25 µM), was added together with FGL_d in some of the set-ups.

In hippocampal neuronal cultures, FM 1-43 staining was used to evaluate pre-synaptic function (described above). Cell viability in both primary cell cultures and OHSC was measured using the MTS [3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide] assay [Malich et al, 1997]. In, addition, cell viability in OHSC was measured using propidium iodide (PI) staining [Laake et al, 1999].

Results

- 5 The metabolic activity of dissociated hippocampal cell cultures was significantly reduced after 1 hour (31%) and 4 hours (30%) following 20 min of OGD, compared to control cultures, as measured by the MTS assay. When cell cultures were treated with FGL_d (24 hours before or immediately after OGD) the effect of OGD on metabolic activity was diminished. The metabolic activity in OHSC showed a different
10 response to OGD. One hour after OGD, the metabolic activity was significantly increased (19%) compared to control, while a longer period of reoxygenation (24 hours) resulted in a decrease (24%) of metabolic activity. At both time-points, treatment with FGL_d 24 hours prior to OGD caused a normalization of the metabolic activity in the hippocampal slices, compared to the control. In both the dissociated cell
15 cultures and OHSC, FGL_d treatment alone led to an increase in metabolic activity. In addition, pretreatment with FGL_d for 24 hours did not diminish the OGD-induced reduction in metabolic activity if the FGFR-inhibitor SU 5402 was present together with FGL_d, indicating that FGL_d might influence metabolic activity through FGFR activation.
20 In dissociated hippocampal cell cultures, the rate of pre-synaptic vesicle release (FM 1-43 destaining) upon high potassium stimulation was statistically significantly enhanced (from 0.04 sec⁻¹ to 0.06 sec⁻¹) by FGL_d treatment and decreased after OGD when analyzed at 1 h. and 4 h (both from 0.04 sec⁻¹ to 0.02 sec⁻¹). If cell cultures
25 were treated with FGL_d either 24 hours before or immediately after OGD, the OGD-induced decrease in the rate of FM 1-43 release was diminished. Furthermore, the FGFR-inhibitor SU 5402 abrogated the effect of FGL_d on the OGD-induced decrease in FM 1-43 release.
In OHSC, 10 minutes of OGD induced delayed cell damage as estimated by
30 propidium iodide (PI) staining. There was a significant increase in the PI staining of OHSC (% of total CA1 area) at both 4 hours (0.3% to 5.0%) and 24 hours (0.6% to 23.1%) after OGD. This effect could be almost completely abolished by pre-treating the slices for 24 hours with FGL_d. Cell damage was also avoided by treating slices with FGL_d immediately after OGD, but not if FGL_d treatment was delayed until 4
35 hours after OGD. The control peptide FGL_c did not affect cell damage induced by

OGD, and if slices were pre-incubated with the FGFR-inhibitor SU 5402 together with FGL_d, the PI staining was similar to that of OGD alone.

Discussion

5 The FGL_d peptide has neuroprotective effects when applied 24 hours before or immediately after a transient deprivation of oxygen and glucose in two different *in vitro* model systems. The OGD caused a reduction in metabolic activity in dissociated hippocampal cell cultures and in OHSC, and in both cases treatment with FGL_d could partially or completely normalize metabolic activity after OGD.

10 Ischemia has previously been reported to cause either enhanced synaptic transmission [Jourdain et al., 2002] or reduced synaptic transmission [Bolay et al., 2002], depending on the model used and the severity of the ischemic challenge. In the model used in this study, 20 minutes of OGD caused a reduction in presynaptic vesicle release upon potassium stimulation, and this reduction was attenuated by 15 administering the FGL_d peptide either 24 hours before or immediately after OGD.

20 Thus, for all three parameters investigated (metabolic activity, presynaptic vesicle release and cell viability), the FGL peptide could partially or completely inhibit the effects of OGD. Furthermore, this neuroprotective ability of FGL could in all three cases be inhibited by the FGFR-inhibitor SU 5402, indicating that FGFR activity is necessary for the action of FGL.

25 **Example 7. Effect of FGL on social behaviour of rats and β-amyloid induced neurotoxicity in vivo.**

Description of the model

The present study employed a rat model, in which neurotoxicity and cognitive impairment were induced by the (25-35) β-amyloid peptide fragment [Maurice T., et al. (1996) and Delobette S., et al. (1997)]. Short-term memory deficit induced by the 30 (25-35) β-amyloid peptide fragment in rat is of special importance to study, as it resembles one of the clinical manifestations of Alzheimer's disease. Short-term memory deficit in rats was evaluated using three different behavioural tests: the Social Recognition [Kogan et al, 2000], Open Field [Cerbone and Sadile, 1994; Vianna et al, 2000] and Y-maze [Clayton & Williams, 2000] tests. In addition, neuropathologi-

cal investigations were performed on brain tissue collected at the time of sacrifice. The FGL peptide was administered either sub-occipitally or intranasally.

Methods

- 5 The timeline of a representative social-recognition study with early treatment is schematically shown in Figure 26 (A).
Following an acclimatisation period of five days, rats were treated with the (25-35) β -amyloid peptide fragment (A- β) (25 μ g per animal), which was injected i.c.v. in the right lateral ventricle of the brain (day 0).
- 10 The FGL peptide (either FGL_L or FGL_d) was administered sub-occipitally (5 μ g per animal per administration) or intranasally (400 μ g per animal per administration) at days 7, 10 and 13 or at days 30, 33 and 36. Behaviour of animals was evaluated using the Social Recognition test, the Open Field test or the Light-dark Discriminating Y-maze. On day 28-30 or day 51 after i.c.v. administration of the (25-35) β -amyloid fragment, the animals were sacrificed, and in selected studies, the brain tissue was collected, histological sections were immunostained with A- β specific antibodies and the amyloid-burden was calculated as a percentage of the A- β positive area. In addition, the number of neurons in selected areas of brain-sections was calculated using a stereological approach.

20

Effects of (25-35) A- β /Vehicle-administration on social behaviour and memory

- In the Social Recognition test, an increase in the time spent exploring a juvenile rat during the second trial was detected in rats that received the (25-35) β -amyloid fragment as compared to the control animals receiving vehicle (V). In the Open Field test, (25-35) β -amyloid fragment-treated rats did not decrease exploratory activity during a 20-minute session, in contrast to the decrease noted in the group of control animals. In the Light-dark Discriminating Y-maze with positive food reinforcement, rats treated with the (25-35) β -amyloid fragment demonstrated an increase in the number of errors (entries into the wrong arms).
- 25 Thus, in all three tests, i.c.v. administration of the (25-35) β -amyloid fragment resulted in a cognitive deficit displayed as short-term memory impairments.

Effects of FGL administration on behaviour and memory of rats in which cognitive impairment was induced by i.c.v.-administration of the (25-35) β-amyloid fragment:

Social Recognition test and Neuro-histopathology

- Study 1: FGL was administered sub-occipitally at days 7, 10 and 13 following i.c.v. administration of the (25-35) β-amyloid fragment. No effect of FGL_d was observed in the Social Recognition test at day 14, the day after the last administration of FGL_d and no significant effect was observed in the neuro-histopathology evaluation of the CA3 area of hippocampus comparing placebo-treated rats with rats receiving either the (25-35) β-amyloid fragment alone or with FGL_d.
- Study 2: FGL was administered sub-occipitally at days 7, 10 and 13 following i.c.v. administration of the (25-35) β-amyloid fragment. Administering the (25-35) β-amyloid fragment resulted in a statistically significant increase in the time spent by the rat exploring a juvenile animal at a second meeting as well as in the amyloid burden and neuronal cell death when compared to control rats. However, treatment with FGL_L and FGL_d significantly reduced the increased time spent by the treated animal exploring a juvenile rat during the second trial, when compared to the animals treated only with the β-amyloid peptide fragment. Treatment with FGL_L and FGL_d also significantly reduced the amyloid burden in the cingulate cortex (from 100% to 39% & 42%, respectively) and in the CA3 area of the hippocampus (from 100% to 17% & 44%, respectively). In addition, FGL_L and FGL_d caused an increase in the density of neurons (from 15 to 21 & 22 neurons/mcm²×10⁻⁴, respectively) in the CA3 zone of the hippocampus, when compared to the group of rats treated only with the (25-35) β-amyloid fragment. The results are graphically presented in Figure 13-16.
- Study 3: FGL_L was administered sub-occipitally at days 30, 33 and 36 following i.c.v. administration of the (25-35) β-amyloid fragment. A statistically significant reduction was observed at day 44, one week after the last administration of FGL_L, in the ratio of the time the rats spent investigating a novel object (juvenile rat) on the second exposure compared to the (25-35) A-β/Vehicle treated control rats (from 0.50 to 0.41), when FGL_L was administered sub-occipitally. In addition, neuropathology investigations showed a statistically significant reduction in

amyloid burden (from 100% to 24%) in the cingulate cortex and the CA3 area of Hippocampus (from 100% to 29%). In addition, a statistically significant decrease in the percentage of damaged neurons (from 27 to 21 neurons/ $\text{mm}^2 \times 10^{-4}$) was observed in the cingulate cortex, compared to the group of rats treated only with the (25-35) β -amyloid fragment. The results are graphically presented in Figures 17-20.

- 5
- o Study PR-1011-FGL-NI: FGL_d was administered intranasally at days 7, 10 and 13 following i.c.v. administration of the (25-35) β -amyloid fragment. Treatment 10 with the Amyloid beta peptide induced a short term memory deficit, which was abrogated by treatment with FGL-peptides. In the cingulate cortex, a statistically significant increase in the neuronal density (from 11 to 14 $\mu\text{m}^2 \times 10^{-4}$) was observed in response to FGL_d administration, compared with the group of rats treated only with the (25-35) β -amyloid fragment. The results are graphically 15 presented in Figures 21-23.

Open Field study

The rats that received FGL_d administered sub-occipitally at days 7, 10 and 13, following i.c.v.-administration of the (25-35) β -amyloid fragment, showed a statistically 20 significant decrease in the time spent exploring holes (from 3.3 s to 0.1 s) and rearing (from 7.0 s to 0.7 s) in the last measurement period, compared with the first measurement period at day 14, the day after the last administration of FGL. The group of rats treated with only the (25-35) β -amyloid fragment showed no statistically significant difference between the two measurement periods. The locomotion 25 activity was unaffected at day 14.

Light-dark Discriminating Y-maze study with positive food-reinforcement

The rats that received FGL_d administered sub-occipitally showed a statistically significant reduction (from 3.8 to 1.7) in the number of errors at day 25, the last day of 30 training, while no significant differences were observed at training days 21-24 in comparison with the (25-35) β -amyloid fragment-treated control rats.

Discussion

The model used in this study, the (25-35) β -amyloid fragment induced neuro-toxicity, reflects many aspects of neuro-pathology and cognitive impairment seen in Alzheimer's patients. In this model, i.c.v.-administration of the (25-35) β -amyloid peptide fragment results in deposition of the β -amyloid peptide, starting at day 14 after the (25-35) β -amyloid peptide fragment administration, which lead to neuronal cell death and cognitive impairment (short-term memory deficit).

5 The experiments of early administration of the FGL peptide (at days 7, 10 and 13 after the i.c.v.-injection of the (25-35) β -amyloid fragment) clearly demonstrate that
10 both sub-occipital and intranasal administration of FGL results in prevention of short-term memory deficit, a decrease of β -amyloid burden in the various brain areas and a decrease in neuronal cell death. The administration of the FGL peptide at a later stage, at days 30, 33 and 36, after i.c.v.-injection of the (25-35) β -amyloid peptide fragment ameliorated both the neuropathological changes in the brain tissue and the
15 memory deficit.

20 The important aspect of these studies was to compare the effectiveness of different routes of FGL administration. The results obtained clearly indicate that the intranasal administration of the FGL peptide effectively reduced the memory deficit induced by treatment with the (25-35) β -amyloid peptide fragment, although the potency of the FGL peptide administered intranasally was much lower, compared to the potency of the peptide administered sub-occipitally. It should be noted that the effect of intranasally administered FGL on memory was dose-dependent.

25 In conclusion, taken together, these results indicate that the FGL peptide has the potential to reduce neuropathology and to reduce the memory deficits induced by treatment with the (25-35) β -amyloid peptide fragment. These findings also indicate that the FGL peptide has a therapeutic potential for the treatment of cognitive deficits associated with β -amyloid deposition in patients with Alzheimer's Disease.
30

Example 8. Sensory Motor Control Development in Rat Pups

The neonatal CNS of immature rodents is a useful model for studying drugs influencing the maturation of brain functions. The maturation of postural adjustment (lo-

comotion) needs integration of motor and sensory systems. The maturation of the postural and locomotor functions during the first post-natal week is connected with the growth of the descending pathways from brainstem and cortico-spinal tracts, reaching the thoracic levels at post-natal day (PND) 3 and the lumbar level at PND 6 [Clarac et al., 1998]. Thus, the period between PND 3 and PND 6 can be considered as a period sensitive to the effects of agents able to modify post-natal integration of the sensory-motor system. A number of motor control tests are available, which can be used for determining the effect of neuro-active drugs such as FGL. In the present study, the Surface Righting Response performance [Tilney, 1933], the Pivoting Locomotion performance [Altman et al, 1975], the Negative Geotaxis performance [Henck et al, 2001], and the Forepaw Suspension performance [Kimler et al, 1998] of rat pups were chosen to evaluate the effect of FGL on sensory motor control development. The time-course of the experiments is schematically presented in Figure 26 (B).

15

Dose-Response study. The effect of intranasal administration of 0.026, 0.26 and 2.6 µg FGL_d per day at post-natal day (PND) 1, 2 and 3 on performance of the Surface Righting Reflex by rat pups was evaluated. A total of five litters, each consisting of 10 pups (five males and five females) were used in this study.

20

Administration of FGL_d significantly improved performance of the Surface Righting Reflex at PND 5 (from 3.4 s to 1.3 s) administered at a concentration of 2.6 µg per pup per administration compared to the vehicle-treated control animals.

25

Sensory Motor Control Development study. The effect of FGL on sensory motor control development (Surface Righting Reflex, Negative Geotaxis Reflex, Pivoting Locomotion and Forepaw Suspension performances) of rat pups receiving 2.6 µg peptide intranasally daily at PND 1, 2 and 3 was evaluated. A total of six litters, each consisting of 10 pups (five males and five females), were used in the study.

30

Administration of FGL_L statistically significantly improved performance of the Surface Righting reflex at PND 4 (from 2.1 s to 1.6 s) (Figure 24) and of the Negative Geotaxis Reflex at PND 6 (from 56.7% to 80.0%) (Figure 25), compared with the vehicle-treated control rats, without affecting the Pivoting Locomotor activity or the Forepaw Suspension performance. This indicates that FGL promotes the post-natal

sensory-motor control development, without disturbing other functions. In addition, no significant effect on Negative Geotaxis performance was observed at PND 9, suggesting that the motor control development was already completed by PND 9.

Discussion

5 The present results demonstrate an effect of the FGL peptide in modulation of the initial processes of CNS plasticity during post-natal development, and suggest that the FGL peptide, by stimulating the FGF receptor, may have a potential to accelerate the development of sensori-motor functions.

10 **Example 9. The Contextual Fear Conditioning Test and the Morris Water Maze Test**

The Contextual Fear Conditioning test (CFC) test [Cordero et al, 2003] and the Morris Water Maze (MWM) test [Morris, R., 1984] are tasks that assess learning and memory, and are used to compare the performance of normal control rats and of 15 rats treated with drugs.

In the Contextual Fear Conditioning test, rats experienced inescapable shocks when exposed to a *new environment, which results in the development of a conditioned fear response, consisting of a characteristic immobility or 'freezing response', when subsequently re-exposed to the same environment [Blanchard and Blanchard, 20 1969].

In the Morris Water Maze test, rats are evaluated in the spatial training task to find a hidden platform in order to escape swimming in the water.

25 The present study investigated whether post-training administration of the FGL_d peptide is effective in modulating the memory consolidation in aversive and/or new situations.

The Contextual Fear Conditioning test

The rats were handled for 120 seconds every day for 3 days preceding the first day of the experiment. On day 1 (training), the rats received a 1-second shock (unconditioned stimuli) each minute for two minutes. Animals were injected immediately after 30 training with 5 µl FGL_d (5 µg), the control peptide (5 µg) or the vehicle. Each treatment group consisted of 11-14 rats. In total, 39 animals were evaluated. Animals were tested on days 2, 7 and 30 by placing them in the chamber used for condition-

ing, but in the absence of a shock, for 8 minutes. Each rat was rated as either "freezing" or "active", every 2 seconds, using a time-sampling procedure.

The time-course of the experiments is schematically presented in Figure 26 (C).

- 5 A statistically significant increase in percentage of freezing time after FGL_d administration was detected when animals were tested on day 2, (from 50.5% to 73.5%) and day 7 (from 30.0% to 60.3%) after conditioning compared to rats receiving placebo or the control. This indicates that FGL_d is capable of improving the long-term retention of the conditioned fear response when administered post-training in adult male Wistar rats.

The Morris Water Maze test

- In the Morris Water Maze study, rats were pre-trained to locate a hidden platform by daily training for 120 seconds for five days (days -4 to 0) before the experiment. On 15 days 1 and 2, rats were given 3 consecutive trials in which the platform location remained constant. The time during which the rats were able to locate the platform was recorded within a maximum test-period of 120 seconds. The rats received a 5 µl injection of the FGL_d peptide (5 µg), the control peptide (5 µg), or the vehicle solution immediately after each training trial on days 1 and 2.

- 20 The animals were tested 24 hours (day 3) and 7 days (day 10) after training. Fourteen days after training (day 17), the location of the platform was changed. Rats were trained to find a new location of the platform in three consecutive trials ("reversal learning"). In total, 48 animals were evaluated. Animals were divided into three 25 groups consisting of 12-18 rats per treatment group.

- 30 Administering FGL_d improved consolidation of long-term memory in rats as evaluated in the Morris Water Maze test. At day 2, the day after the first administration of FGL_d, FGL_c or vehicle, the FGL_d-treated rats were able to locate the platform statistically significantly faster (65.8 s) than the untreated rats (93.0 s). The effect was also statistically significant at day 17, suggesting an improved learning ability.

The time-course of the experiment is schematically presented in Figure 26 (D).

Additional experiments in the Open Field test showed that FGL_d did not influence emotional or locomotor behaviour in rats.

Discussion

- 5 The results of the CFC/MWM studies demonstrated a long-term effect of FGL_d on learning and memory of normal rats. FGL may be acting by triggering signalling cascades involved in long-term memory formation. As it is shown above, FGL can modulate synaptic plasticity acting through the FGFR, which results in an increased pre-synaptic vesicle turnover. Together, these results suggest that FGL has a very
10 good therapeutic potential in the treatment of patients with learning and memory impairment.

Example 10. In vivo pharmaco-kinetics of FGL_L: estimation of FGL_L in plasma and CSF

- 15 The levels of the administered FGL_L in rat and dog plasma and CSF were assessed by the radio-immuno assay.

Assay

- a) Rat and dog plasma samples contained lithium heparin as an anti-coagulant.
20 b) FGL_L was iodinated. Iodination was done by using the Iodogen method. The iodinated peptide was purified from unincorporated I¹²⁵ on C18 column.
c) The measurements were done in replicates.

- 25 1. Plasma (or CSF) sample (20 µl) was added to EDTA containing tubes (n=2). Non-specific binding (NSB) tubes containing non-immune rabbit serum (or CSF from control animals) (20 µl) were used as control.
- 30 2. Diluted radiolabelled FGL_L (100 µl; approximately 20,000 cpm/tube of tracer) was added. Control tubes containing the tracer only were also prepared.
3. Diluted antisera sample (1: 20,000 for PAb268; 100 µl) was added to all tubes (except the control tubes. The contents were vortex mixed).
4. The tubes were incubated overnight at 4°C.
5. Anti-rabbit SAC-Cell solution (0.1 ml) was added to all tubes except the tubes containing the tracer. The contents were vortex mixed and then incubated at room temperature for 30 min.

6. Wash buffer (2 ml) was added to all tubes except the tubes containing the tracer and the tubes were centrifuged at 3000 rpm for 5 minutes.
7. The supernatant was decanted and the tubes were left to drain for no more than 1 minute.
- 5 8. The wash buffer (1.5 ml) was added to the tubes again and the tubes were centrifuged at 3000 rpm for 5 minutes.
9. The supernatant was decanted and the tubes were left to drain for no more than 1 minute. The tubes were centrifuged at 3000 rpm for 1 minute thereafter.
- 10 10. All tubes were counted two times: first for 3 minutes and for 10 min.

Results

A calibration curve precision profile was prepared in replicate (n=6) in rat plasma over the concentration range 0.080 ng/ml to 20.48 ng/ml of FGL_L. Good precision was observed over the whole concentration range with acceptable accuracy (<25%) down to 0.160 ng/ml, which gives an indication of the potential lower limit of quantification (LLOQ) of the assay in rat plasma. Increasing the counting time did improve the precision of the assay.

A calibration curve precision profile was prepared in replicate (n=6) in dog plasma over the concentration range 0.080 ng/ml to 20.48 ng/ml.

Pharmacokinetic parameters were calculated using the computer program WinNon-lin Pro version 3.3 (Pharsight Corporation, USA). Values that were below the limit of quantification (BLQ) were entered as zero in the calculation of the mean.

There were also defined some toxicokinetic parameters. The toxicokinetic parameters that were measured included: maximum plasma concentrations of FGL_L (C_{max}), their times of occurrence (T_{max}), and plasma concentrations at 24 hours post-dose (C_{24}). Areas under the plasma FGL_L concentration-time curves within a 24-hour dosing interval (AUC_{24}) were estimated by the linear trapezoidal rule. In the calculation of AUC_{24} values following intranasal administration, the times of the samples were relative to the start of administration (assuming a 10-minute break between each of the 5 administration procedures). Terminal rate constants (k) were estimated by fitting a linear regression of log concentration against time. Terminal half-lives ($t\frac{1}{2}$) were calculated as $\ln 2/k$.

FGL_L levels in the CSF from the two rats treated intravenously with 50 mg/kg were 321 and 269 ng/ml. FGL_L levels in the CSF from other animals treated subcutaneously with 100 mg/kg were 242, 92 and 81 ng/ml. These data provide evidence that FGL_L enters the CNS when administered parenterally.

5

Toxicokinetic samples taken after the first dose revealed:

Combined male and female exposures (50 mg/kg/day,i.v.):

$$\text{AUC} = 51,026 \text{ h.ng/mL}; C_{\max} = 20,951 \text{ ng/mL}.$$

Combined male and female exposures (100 mg/kg/day,s.c.):

10 $\text{AUC} = 50,119 \text{ h.ng/mL}; C_{\max} = 8,346 \text{ ng/mL}.$

Table 12 shows the toxicokinetic parameters calculated in dogs receiving doses of FGL_L of 15, 30 and 75 mg/kg, s.c.

Dose	Dog No	Day	C _{max} (ng/ml)	t _{max} (hr)	AUC(0-24) (hr*ng/ml)	AUC (hr*ng/ml)
15 mg/kg	2	1	7132	2.0	62965	84829
		7	2204	2.0	41531	70419
30 mg/kg	3	1	9863	2.0	81243	104608
		7	2709	4.0	38208	58518
75 mg/kg	4	1	9222	4.0	13049 7	161489
		7	2281	8.0	40642	63191

- 15 Mean levels of FGL_L in the CSF from the three dogs (sampled one hour after the last dose at day 8) ranged from 4.1 ng/ml for animal No. 2 to 8.1 ng/ml for animal No. 3, indicating penetration of FGL_L into the CNS, following subcutaneous dosing.

Claims

1. A compound comprising at least two peptide sequences of the formula

5 L1-A-L2-B-L3-C-L4-D-L5

wherein

one of A, B, C, D is selected from a hydrophobic amino acid residue,

one of A, B, C, D is selected from a basic amino acid residue or Ser, Thr, Asn or Gln,

10 one of A, B, C, D is selected from an acidic amino acid residue or Ser, Thr, Asn or Gln,

one of A, B, C, D is Gly or Ala, and

L1, L2, L3, L4 and L5 is selected from a chemical bond or an amino acid sequence having n amino acid residues, wherein n is an integer of from 0 to 5,

15 wherein

said peptide sequences are connected to each other through a linker having the formula

X[(A)nCOOH][(B)mCOOH]

20

n and m independently are an integer of from 1 to 20,

X is HN, H₂N(CR₂)pCR, RHN(CR₂)pCR, HO(CR₂)pCR, HS(CR₂)pCR, halogen-(CR₂)pCR, HOOC(CR₂)pCR, ROOC(CR₂)pCR, HCO(CR₂)pCR, RCO(CR₂)pCR, [HOOC(A)n][HOOC(B)m]CR(CR₂)pCR, H₂N(CR₂)p, RHN(CR₂)p, HO(CR₂)p,

25 HS(CR₂)p, halogen-(CR₂)p, HOOC(CR₂)p, ROOC(CR₂)p, HCO(CR₂)p, RCO(CR₂)p, or [HOOC(A)n][HOOC(B)m](CR₂)p, wherein p is 0 or integer of from 1 to 20,

A and B independently are a substituted or unsubstituted C₁₋₁₀ alkyl, a substituted or unsubstituted C₂₋₁₀ alkenyl, a substituted or unsubstituted cyclic moiety,

30 a substituted or unsubstituted heterocyclic moiety, a substituted or unsubstituted aromatic moiety, or A and B together form a substituted or unsubstituted cyclic moiety, substituted or unsubstituted heterocyclic moiety, substituted or unsubstituted aromatic moiety.

2. The compound according to claim 1, wherein at least one of the two peptide sequences is a ligand for a functional cell surface receptor.
- 5 3. The compound according to claim 2, wherein the functional cell surface receptor is a receptor selected from the family of fibroblast growth factor receptors (FGFRs) comprising FGFR1, FGFR2, FGFR3 and FGFR4, or functional homologues thereof.
- 10 4. The compound according to claim 2, wherein the at least one of the two peptide sequences is derived from the sequence of a polypeptide selected from the group comprising cell adhesion molecules, cell-surface receptors, heparan sulphate proteoglycans, and metalloproteases; extracellular matrix molecules or growth factors.
- 15 5. The compound according to the claim 4, wherein the cell adhesion molecule is selected from the group comprising
 - Neural Cell Adhesion Molecule (NCAM) (Swiss-Prot Ass. Nos: P13591, P13595-01, P13595),
 - Neural cell adhesion molecule L1 (Swiss-Prot Ass. Nos: Q9QYQ7, Q9QY38, P11627, Q05695, P32004),
 - Neural Cell Adhesion Molecule-2 (NCAM-2) (Swiss-Prot Ass. No: P36335)
 - Neuron-glia Cell Adhesion Molecule (Ng-CAM) (Swiss-Prot Ass. No: Q03696; Q90933),
 - Neural cell adhesion molecule CALL (Swiss-Prot Ass. No: O00533),
 - Neuroglian (Swiss-Prot Ass. No: P91767, P20241),
 - Nr-CAM (HBRAVO, NRCAM, NR-CAM 12) (Swiss-Prot Ass. Nos: Q92823, O15179, Q9QVN3
 - Axonin-1/TAG-1 (Swiss-Prot Ass. Nos: Q02246, P22063, P28685),
 - Axonal-associated Cell Adhesion Molecule (AxCAM) (NCBI Ass. No: NP_031544.1; Swiss-Prot Ass. No: Q8TC35),
 - Myelin-Associated Glycoprotein (MAG) (Swiss-Prot Ass. No: P20917),
 - Neural cell adhesion molecule BIG-1 (Swiss-Prot Ass. No: Q62682),
 - Neural cell adhesion molecule BIG-2 (Swiss-Prot Ass. No: Q62845),
 - Fasciclin (FAS-2) (Swiss-Prot Ass. No: P22648),
 - 20 - Neural cell adhesion molecule HNB-3/NB-3 (Swiss-Prot Ass. Nos: Q9UQ52,
- 25
- 30
- 35

P97528, Q9JMB8)

- Neural cell adhesion molecule HNB-2/NB-2 (Swiss-Prot Ass. Nos: O94779, P07409, P97527),
- Cadherin (Swiss-Prot Ass. No: Q9VW71),
- 5 - Junctional Adhesion Molecule-1 (JAM-1) (Swiss-Prot Ass. Nos: Q9JKD5, O88792),
- Neural cell adhesion F3/F11(Contactin) (Swiss-Prot Ass. Nos: Q63198, P1260, Q12860, Q28106, P14781, O93250),
- Neurofascin (Swiss-Prot Ass. Nos: Q90924, Q91Z60; O42414),
- 10 - B-lymphocyte cell adhesion molecule CD22 (Swiss-Prot Ass. Nos: Q9R094, P20273),
- Neogenin (NEO1) (Swiss-Prot Ass. Nos: Q92859, P97603, Q90610, P97798),
- Intercellular Cell Adhesion Molecule-5 (ICAM-5/telencephalin) (Swiss-Prot Ass. Nos: Q8TAM9, Q60625) or
- 15 - Galactose binding lectin-12 (galectin-12) (Swiss-Prot Ass. Nos: Q91VD1, Q9JKX2, Q9NZ03),
- Galactose binding lectin-4 (galectin-4) (Swiss-Prot Ass. No: Q8K419; P38552), or fragments, or variants thereof.

- 20 6. The compound according to the claim 4, wherein the functional cell-surface receptor is selected from the group comprising
- Fibroblast Growth Factor Receptor 1 (FGFR1) (Swiss-Prot Ass. Nos: Q9QZM7, Q99AVV7, Q9UD50, Q63827),
 - Fibroblast Growth Factor Receptor 2 (FGFR2) (Swiss-Prot Ass. Nos: Q96KM2, P21802, Q63241),
 - 25 - Fibroblast Growth Factor Receptor 3 (FGFR3) (Swiss-Prot Ass. Nos: Q95M13, AF487554, Q99052),
 - Fibroblast Growth Factor Receptor 4 (FGFR4) (Swiss-Prot Ass. No: Q91742),
 - Neurotrophin Tyrosin Kinase Type-2 (NTRKT-2) (Swiss-Prot Ass. No: Q8WXJ5),
 - 30 - Leukocyte Antigen Related Protein-Tyrosine Phosphatase (LAR-PTPRF) (Swiss-Prot Ass. Nos: Q9EQ17, Q64605, Q64604, Q9QW67, Q9VIS8 P10586),
 - Nephrin (Swiss-Prot Ass. Nos: Q925S5, Q9JIX2, Q9ET59, Q9R044, Q9QZS7, Q06500),

- Protein-Tyrosine Phosphatase Receptor type S (PTPRS) (Swiss-Prot Ass. Nos: Q64699, Q13332, O75870),
- Protein-Tyrosine Phosphatase Receptor type kappa (R-PTP-kappa) (Swiss-Prot Ass. No: Q15262),
- 5 - Protein-Tyrosine Phosphatase Receptor type D (PTPRD) (Swiss-Prot Ass. Nos: Q8WX65, Q9IAJ1, P23468, Q64487),
- Ephrin type-A receptor 8 (EPHA8/Tyrosine-Protein Kinase Receptor EEK) (Swiss-Prot Ass. Nos: O09127, P29322),
- Ephrin type-A receptor 3 (EPHA8/Tyrosine-Protein Kinase Receptor ETK-1/CEK4) (Swiss-Prot Ass. No: P29318),
- 10 - Ephrin type-A receptor 2 (Swiss-Prot Ass. No: Q8N3Z2)
- Insulin Receptor (IR) (Swiss-Prot Ass. No: Q9PWN6)
- Insulin-like Growth Factor-1 Receptor (IGF-1) (Swiss-Prot Ass. Nos: Q9QVW4, P08069, P24062, Q60751, P15127, P15208)
- 15 - Insulin-related Receptor (IRR) (Swiss-Prot Ass. No: P14616),
- Tyrosine-Protein Kinase Receptor Tie-1 (Swiss-Prot Ass. Nos: O6805, P35590, Q06806),
- Roundabout receptor-1 (robo-1) (Swiss-Prot Ass. Nos: O44924, AF041082, Q9Y6N7),
- 20 - Neuronal nicotinic acetylcholine receptor alpha 3 subunit (CHRNA3) (Swiss-Prot Ass. Nos: Q8VHH6, P04757, Q8R4G9, P32297)
- Neuronal acetylcholine receptor alpha 6 subunit (Swiss-Prot Ass. Nos: Q15825, Q9R0W9)
- Platelet-Derived Growth Factor Receptor Beta (PDGFRB) (Swiss-Prot Ass.
- 25 Nos: Q8R406, Q05030),
- Interleukin-6 Receptor (IL-6R) (Swiss-Prot Ass. No: Q00560),
- Interleukin-23 Receptor (IL-23R) (Swiss-Prot Ass. No: AF461422),
- Beta-common cytokine receptor of IL-3, IL5 and GmCsf (Swiss-Prot Ass. No: P32927)
- 30 - Cytokine Receptor-Like molecule 3 (CRLF1) (Swiss-Prot Ass. No: Q9JM58),
- Class I Cytokine Receptor (ZCYTOR5) (Swiss-Prot Ass. No: Q9UHH5)
- Netrin-1 receptor DCC (Swiss-Prot Ass. No: P43146),
- Leukocyte Fc Receptor-like Protein (IFGP2) (Swiss-Prot Ass. Nos: Q96PJ6, Q96KM2),
35 - Macrophage Scavenger Receptor 2 (MSR2) (Swiss-Prot Ass. No: Q91YK7), or

- Granulocyte Colony Stimulating Factor Receptor (G-CSF-R) (Swiss-Prot Ass. No: Q99062),
or fragments, or variants thereof.
- 5 7. The compound according to the claim 4, wherein the heparan sulphate proteo-glycan is perlecan (Swiss-Prot Ass. No: P98160), or a fragment, or a variant thereof.
- 10 8. The compound according to the claim 4, wherein the metalloprotease is selected from the group comprising
 - ADAM-8 (Swiss-Prot Ass. No: Q05910),
 - ADAM-19 (Swiss-Prot Ass. Nos: Q9H013, O35674),
 - ADAM-8 (Swiss-Prot Ass. No: P78325),
 - ADAM-12 (Swiss-Prot Ass. Nos: O43184, Q61824),
- 15 - ADAM-28 (Swiss-Prot Ass. Nos: Q9JLN6, Q61824, Q9XSL6, Q9UKQ2),
 - ADAM-33 precursor (Swiss-Prot Ass. Nos: Q8R533, Q923W9),
 - ADAM-9 (Swiss-Prot Ass. Nos: Q13433, Q61072),
 - ADAM-7 (Swiss-Prot Ass. Nos: Q9H2U9, O35227, Q63180),
 - ADAM-1A Fertilin alpha (Swiss-Prot Ass. No: Q8R533),
- 20 - ADAM-15 (Swiss-Prot Ass. Nos: Q9QYY0, O88839, Q13444),
 - Metalloproteinase-desintegrin domain containing protein (TECAM) (Swiss-Prot Ass. No: AF163291),
 - Metalloproteinase 1 (Swiss-Prot Ass. Nos: O95204, Q9BSI6),
or fragments, or variants thereof.
- 25 9. The compound according to the claim 4, wherein the extracellular matrix molecule is selected from the group comprising
 - Collagen type VII (Swiss-Prot Ass. No: Q63870),
 - Fibronectin (Swiss-Prot Ass. Nos: Q95KV4, Q95KV5, P07589, Q28377, U42594, O95609, P11276), or
 - Tenascin-R (Swiss-Prot Ass. Nos: Q15568, O00531, Q90995, P10039),
or fragments, or variants thereof.

10. The compound according to the claim 4, wherein the growth factor is Cytokine-like factor-1 (CLF-1) (Swiss-Prot Ass. No:O75462), or a fragment, or a variant thereof.
- 5 11. The compound according to any of the claims 1 to 10, wherein the at least one of the two peptide sequences is a peptide fragment having the amino acid sequence selected from
EVYVVAENQQGKSKA (SEQ ID NO 1),
NIEVVVEAENALGKKV (SEQ ID NO: 2),
10 ATNRQGKVKAFAHL (SEQ ID NO: 3),
RYVELYVVADSQEFQK (SEQ ID NO: 4)
VAENSRGKNVAKG (SEQ ID NO: 5),
GEYWCVAENQYGQR (SEQ ID NO: 6),
RLAALNGKGLGEIS (SEQ ID NO: 7),
15 KYIAENMKAQNVAKEI (SEQ ID NO: 8),
TIMGLKPETRYAVR (SEQ ID NO: 9),
KGLGEISAATEFKT (SEQ ID NO: 10),
NMGIWVQAENALG (SEQ ID NO: 11),
IWVQAENMLG (SEQ ID NO: 12),
20 EIWVEATNRLG (SEQ ID NO: 13),
VVVQAAANALG (SEQ ID NO: 14),
EVWIEKDPAKGRI (SEQ ID NO: 15),
ATNKGGEVKKNGHL (SEQ ID NO: 16),
KYVELYLVDYLEFQK (SEQ ID NO: 17),
25 RYVELYVVVDNAEFQ (SEQ ID NO: 18),
KYVELVIVADNREFQR (SEQ ID NO: 19),
KYIEYYLVLDNGEFKR (SEQ ID NO: 20),
RYLELYIVADHTLF (SEQ ID NO: 21),
KYVEMFVVVNHQRFFQ (SEQ ID NO: 22),
30 RYVELFIVVDKERY (SEQ ID NO: 23),
KYVELFIVADDTVYRR (SEQ ID NO: 24),
KFIELFVVADEYYVYRR (SEQ ID NO: 25),
KIVEKVIVADNSEVRK (SEQ ID NO: 26),
VELVIVADHSEAQK (SEQ ID NO: 27),
35 VAENSRGKNIAKG (SEQ ID NO: 28),

IAENSRGKNVARG (SEQ ID NO: 29),
AENSRGKNSFRG (SEQ ID NO: 30),
IASNLRGRNLAKG (SEQ ID NO: 31),
IPENSLGKTYAKG (SEQ ID NO: 32),
5 IAENMKAQNEAK (SEQ ID NO: 33),
QFIAENMKSHNETKEV (SEQ ID NO: 34),
GEYWCVAKNRVGQ (SEQ ID NO: 35),
GSYTCVAENMVGK (SEQ ID NO: 36),
GKYVCVGTNMVGER (SEQ ID NO: 37),
10 GNYTCVVENEYG (SEQ ID NO: 38),
GEYTCLAGNSIG (SEQ ID NO: 39),
QYYCVAENGYG (SEQ ID NO: 40),
GEYYQEAEQNGYG (SEQ ID NO: 41),
GNYTCLVENEYG (SEQ ID NO: 42),
15 GMYQCLAENAYG (SEQ ID NO: 43),
GMYQCAENTHG (SEQ ID NO: 44),
GIYYCLASNNYG (SEQ ID NO: 45),
GGYYCTADNSYG (SEQ ID NO: 46),
GEYQCFARNDYG (SEQ ID NO: 47),
20 GEYFCLASNMG (SEQ ID NO: 48),
GEYQCFARNKFG (SEQ ID NO: 49),
GEYFCLASNMG (SEQ ID NO: 50),
GGYYCTADNNYG (SEQ ID NO: 51),
GNYSCEAENAWGTK (SEQ ID NO: 52),
25 GEYTCLAENSLG (SEQ ID NO: 53),
GEYECVAENGRLG (SEQ ID NO: 54),
GNYTCVVENKFGR (SEQ ID NO: 55),
GEYTCLAGNSIG (SEQ ID NO: 56),
GEYFCVASNPIG (SEQ ID NO: 57),
30 EYTCIANNQAGE (SEQ ID NO: 58),
GMYQCVAENKHLG (SEQ ID NO: 59),
GEYMCTASNTIGQ (SEQ ID NO: 60),
EYVCIAENKAGEQ (SEQ ID NO: 61),
GDYTLIAKNEYGK (SEQ ID NO: 62),
35 GFYQCVAENEAG (SEQ ID NO: 63),

GKYECVATNSAGTR (SEQ ID NO: 64),
GEYFCVYNNSLG (SEQ ID NO: 65),
GEYECAATNAHGR (SEQ ID NO: 66),
GAYWCQGTNSVGK (SEQ ID NO: 67),
5 GTYSCVAENILG (SEQ ID NO: 68),
RVAAVNGKGQGDYS (SEQ ID NO: 69),
RVAAINCGIGPFS (SEQ ID NO: 70),
AVLNGKGLG (SEQ ID NO: 71),
ALNGQGLGATS (SEQ ID NO: 72),
10 RLAAKNRAGLGE (SEQ ID NO: 73),
RLGVVTGKDLGEI (SEQ ID NO: 74),
TVTGLKPETSYMVK (SEQ ID NO: 75),
TLTGLKPSTRYRI (SEQ ID NO: 76),
TLTGLQPSTRYRV (SEQ ID NO: 77),
15 TLLGLKPDTTYDIK (SEQ ID NO: 78),
TLQGLRPETAYELR (SEQ ID NO: 79),
TLRGLRPETAYELR (SEQ ID NO: 80),
TLMNLRPKTGYSVR (SEQ ID NO: 81),
TVSGLKPGTRY (SEQ ID NO: 82),
20 TISGLKPDTTY (SEQ ID NO: 83),
TLQGLKPDTAY (SEQ ID NO: 84),
LRGLKPWTQYAV (SEQ ID NO: 85),
IDGLEPDTEYIVR (SEQ ID NO: 86),
LQGLKPWTQYAI (SEQ ID NO: 87),
25 TITGLEPGTEYTIQ (SEQ ID NO: 88),
GLKPWTQYAV (SEQ ID NO: 89),
TLASLKPWTQYAV (SEQ ID NO: 90),
LMGLQPATEYIV (SEQ ID NO: 91),
KGMGPMSEAVQFRT (SEQ ID NO: 92),
30 TLTGLKPDTTYDVK (SEQ ID NO: 93),
ISGLQPETSYSL (SEQ ID NO: 94),
TLLGLKPDTTYDIK (SEQ ID NO: 95),
TISGLTPETTYSI (SEQ ID NO: 96),
GNYSCLAENRLGR (SEQ ID NO: 97),
35 GNYTCVVENRVG (SEQ ID NO: 98),

GTYHCVATNAHG (SEQ ID NO: 99),
LSHNGVLTGYLLSY (SEQ ID NO: 100),
NGVLTGVLRY (SEQ ID NO: 101),
NGVLTGYNLRY (SEQ ID NO: 102),
5 NGNLTGYLLQY (SEQ ID NO: 103),
VDENGVLTGYKIYY (SEQ ID NO: 104),
THNGALVGYSVRY (SEQ ID NO: 105),
NGILTEYILKY (SEQ ID NO: 106),
NGILIGYTLRY (SEQ ID NO: 107),
10 THSGQITGYKIRY (SEQ ID NO: 108),
NGKITGYIYYY (SEQ ID NO: 109),
LSHNGIFTLY (SEQ ID NO: 110),
NGILTEYTLKY (SEQ ID NO: 111),
LDPNGIITQYEISY (SEQ ID NO: 112),
15 NGKITGYIYYY (SEQ ID NO: 113),
HLEVQAFNGRGS GPA (SEQ ID NO: 114),
HTV RAYNGAGYGP (SEQ ID NO: 115),
HLSVKAYNSAGTGPS (SEQ ID NO: 116),
HLAVKAYNSAGTGPS (SEQ ID NO: 117),
20 NLEVRAFNSAGDGP (SEQ ID NO: 118),
HTV LAYNSKGAGP (SEQ ID NO: 119),
LRVLVFNGRGDGP (SEQ ID NO: 120),
HIDVSAFNSAGYGP (SEQ ID NO: 121),
HLAELFNGR (SEQ ID NO: 122),
25 LELQSINFLGGQPA (SEQ ID NO: 123),
HFTV RAYNGAGYGP (SEQ ID NO: 124),
HLEVQAFNGRGSQPA (SEQ ID NO: 125),
VIADQPTFVKYL IK (SEQ ID NO: 126),
TIKGLRPGVVYEGQ (SEQ ID NO: 127),
30 TLTELSPSTQYTVK (SEQ ID NO: 128),
TLDDLAPDTTYLVQ (SEQ ID NO: 129),
TVSDVTPHAIYTVR (SEQ ID NO: 130),
II RGLNASTR YLFR (SEQ ID NO: 131),
TLMNLRPKTGYSVR (SEQ ID NO: 132),
35 TLTGLKPGTEYEVR (SEQ ID NO: 133),

GPEHLMPSSTYVAR (SEQ ID NO: 134),
RVTGLTPKKTYEFR (SEQ ID NO: 135),
LTGLKPGTEYEFR (SEQ ID NO: 136),
EVRVQAVNGGGNGPP (SEQ ID NO: 137),

5 LIKWAINDRGE (SEQ ID NO: 138),
VVSIIAVNGREE (SEQ ID NO: 139),
VVSVYAQQNQNGE (SEQ ID NO: 140),
TISLVAEKGRHK (SEQ ID NO: 141),
HLEVQAFNngrgsgpa (SEQ ID NO: 142),

10 HVEVQAFNngrglgpa (SEQ ID NO: 143),
HVEVQAFNngrglgpa (SEQ ID NO: 144),
EFRVRAVNGAGEG (SEQ ID NO: 145), or
VARVRTRLAPGSRLS (SEQ ID NO: 146), or
or a fragment, or a variant, or homologue thereof.

15 12. The compound according to claim 11, wherein the sequence is EVYV-
VAENQQGKSKA (SEQ ID NO: 1).

20 13. The compound according to claim 11, wherein the sequence is NIEVVVEAE-
NALGKKV (SEQ ID NO: 2).

14. The compound according to any of the preceding claims, wherein the compound
comprises at least two different peptide fragments selected from any of the pep-
tide fragments as defined in claim 11.

25 15. The compound according to any of the preceding claims, wherein the compound
comprises at least two identical peptide fragments selected from any of the pep-
tide fragments as defined in claim 11.

30 16. The compound according to claim 15, wherein the peptide fragments are inde-
pendently having the sequence EVYVVAENQQGKSKA (SEQ ID NO: 1).

17. The compound according to claim 15, wherein the peptide fragments are inde-
pendently having the sequence is NIEVVVEAEANALGKKV (SEQ ID NO: 2).

18. The compound according to claim 14, wherein one of the two peptide fragments is having the sequence EVYVVAENQQGKSKA (SEQ ID NO: 1), and the other is having the sequence NIEWWVEAENALGKKV (SEQ ID NO: 2).
- 5 19. The compound according to any of the preceding claims, said compound being obtained by a method for preparing an LPA enabling presentation of sequence(s) as defined in claim 11 comprising the steps of
- (a) providing by solid phase synthesis or fragment coupling ligands comprising desired sequence(s), the ligands being attached to a solid phase,

10 (b) if necessary, deprotecting any N-terminal amino acid groups while the ligand(s) are still attached to the solid phase,

(c) reacting the ligand(s) having unprotected N-terminal groups with an achiral di- tri- or tetracarboxylic acid so as to provide a construct having a ring structure, and

15 (d) cleaving the construct from the solid phase so as to provide an LPA comprising ligands having free C-terminal groups.

20 20. A pharmaceutical composition comprising a compound as defined in claims 1-19.

21. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for treatment of conditions of the central and peripheral nervous system associated with postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression; for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel.

22. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the treatment of postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression..
- 5 23. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the promotion of wound-healing.
- 10 24. Use a compound as defined in claims 1-19 for the manufacture of a medicament for the treatment of cancer
- 15 25. The use according to claim 24, wherein the cancer is any type of solid tumors requiring neoangiogenesis
- 20 26. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the prevention of death of heart muscle cells, such as after acute myocardial infarction, or after angiogenesis
- 25 27. Use a compound as defined in any of claims 1-19 for the manufacture of a medicament for revascularisation.
- 30 28. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the stimulation of the ability to learn and/or the short and/or long-term memory
29. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the prevention of cell death due to ischemia.
- 30 30. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the prevention of body damages due to alcohol consumption.

31. Use a compound as defined in any of the claims 1-19 for the manufacture of a medicament for the treatment of prion diseases.

5 32. Use of a medicament of any of the claims 21-31 for the treatment of a disease or a condition as defined in claims 21-31.

10 33. A method of treatment of an individual in need comprising a step of administering to said individual a compound as defined in any of the claims 1 to 19, and/or a pharmaceutical composition as defined in claim 20, and/or medicament as defined in claims 21-31.

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25 MAJ 2004

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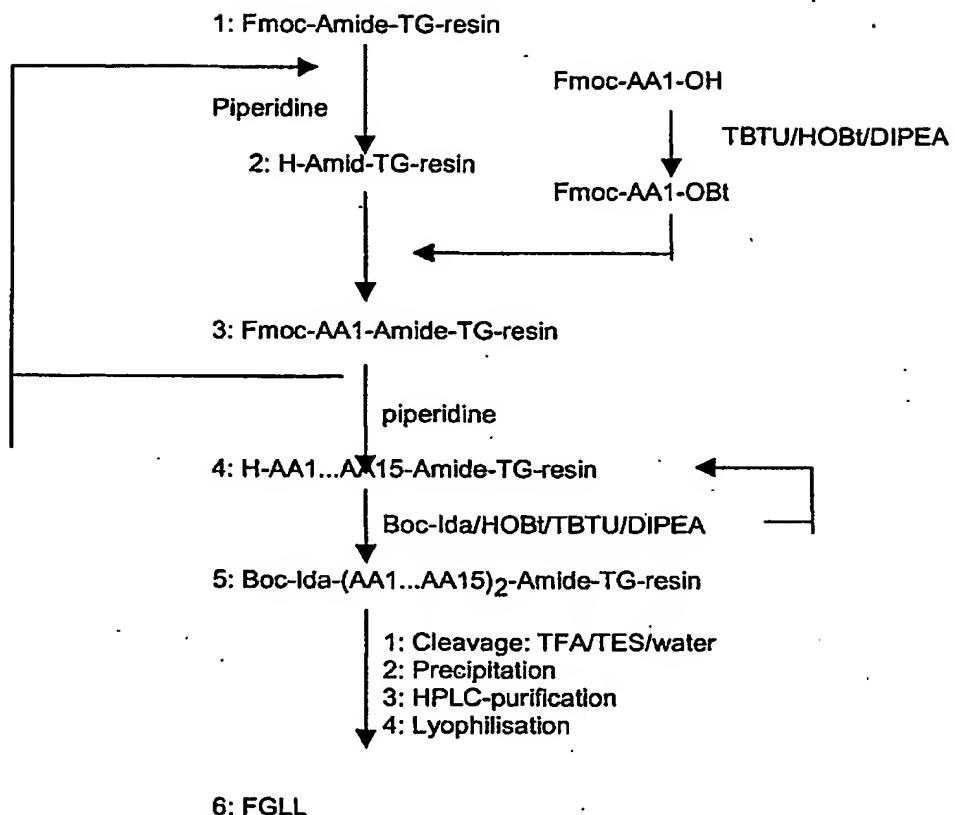


Figure 1

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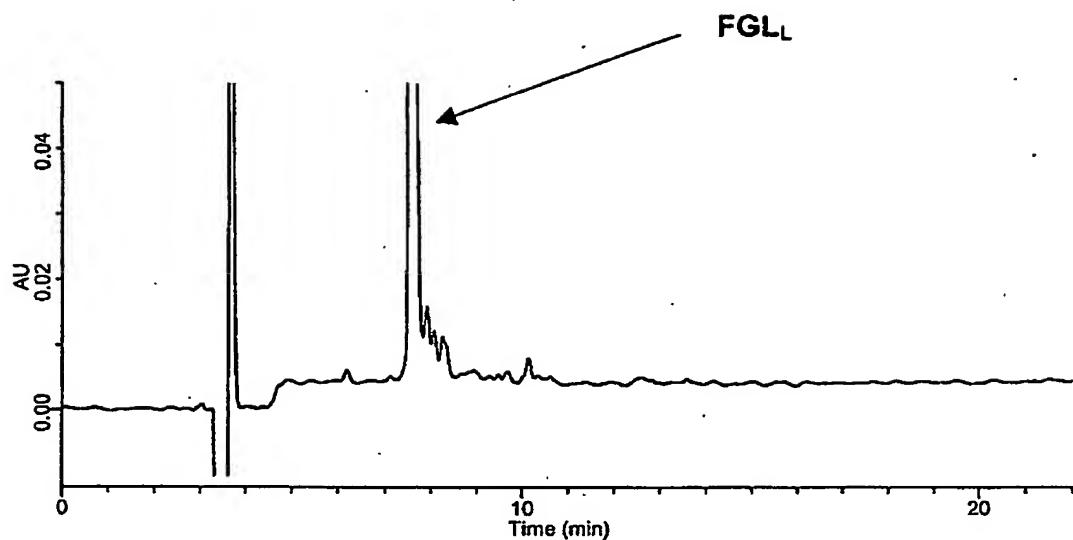


Figure 2

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25 MAJ 2004
PVS

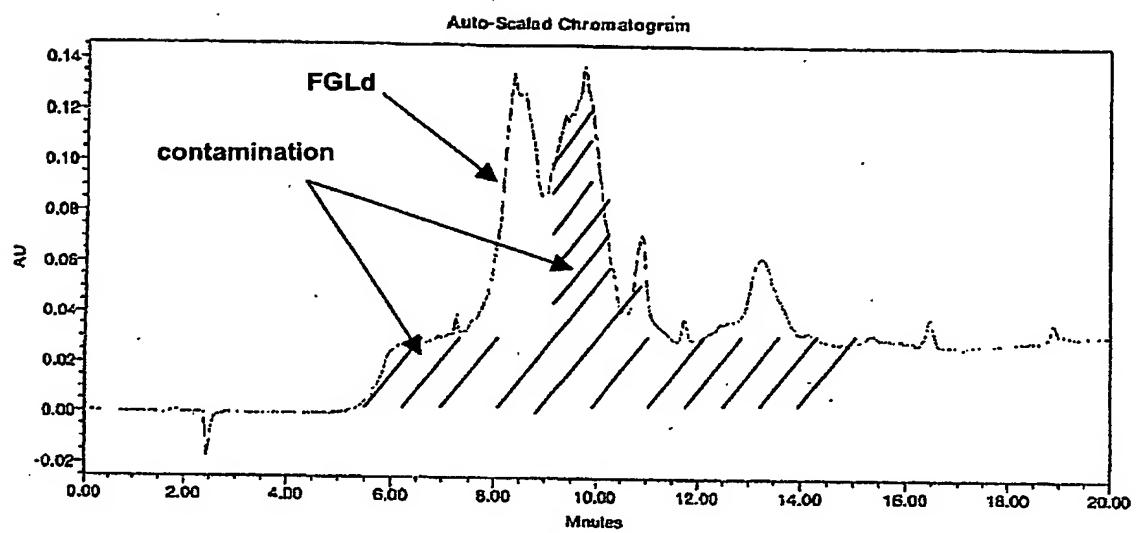


Figure 3

Modtaget
25 MAJ 2004
PVS

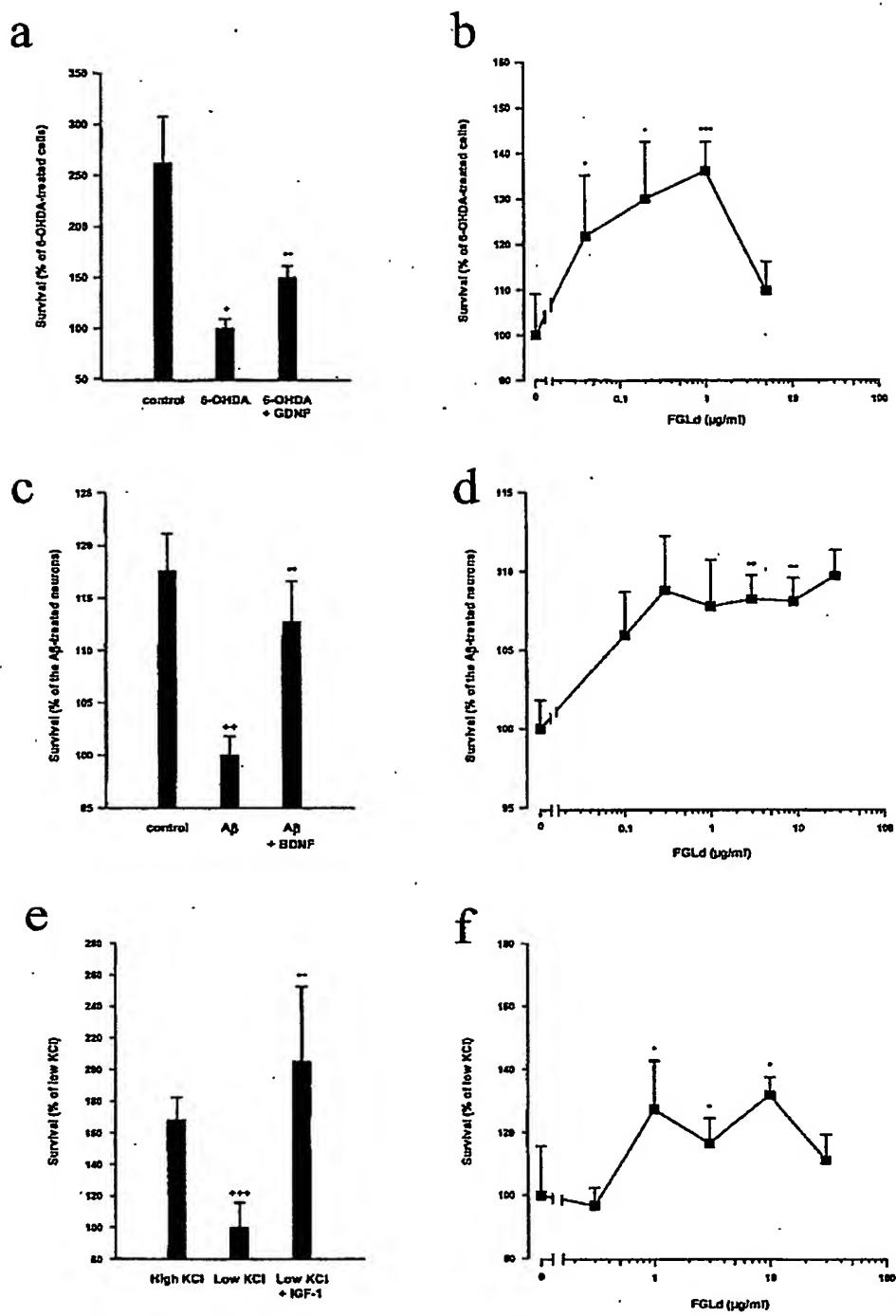


Figure 4

Modtaget
25 MAJ 2004

PVS

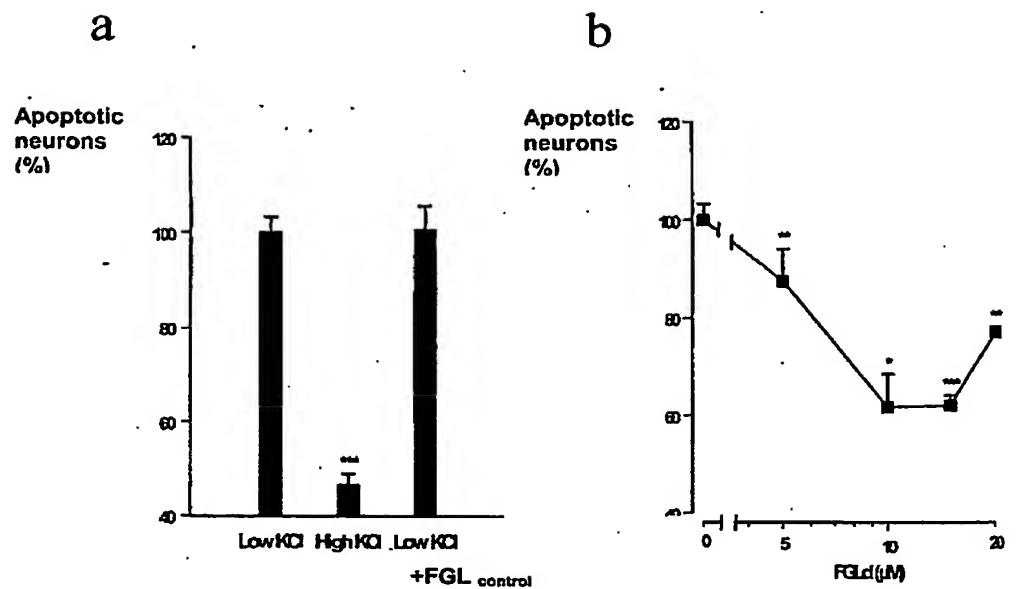


Figure 5

Modtaget
25.11.2004
PVS

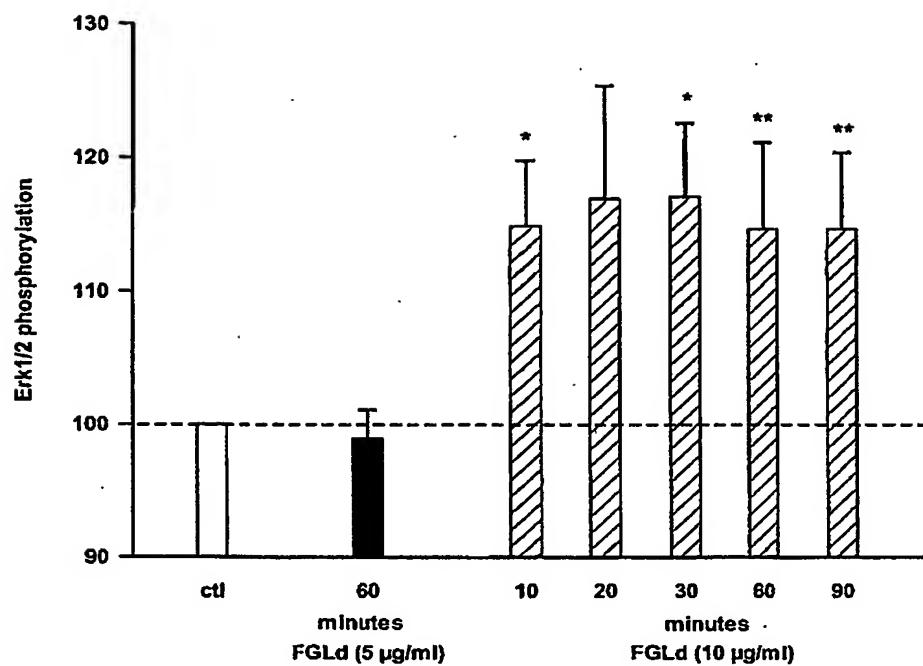


Figure 6

Modtaget
25 MAJ 2004
FVS

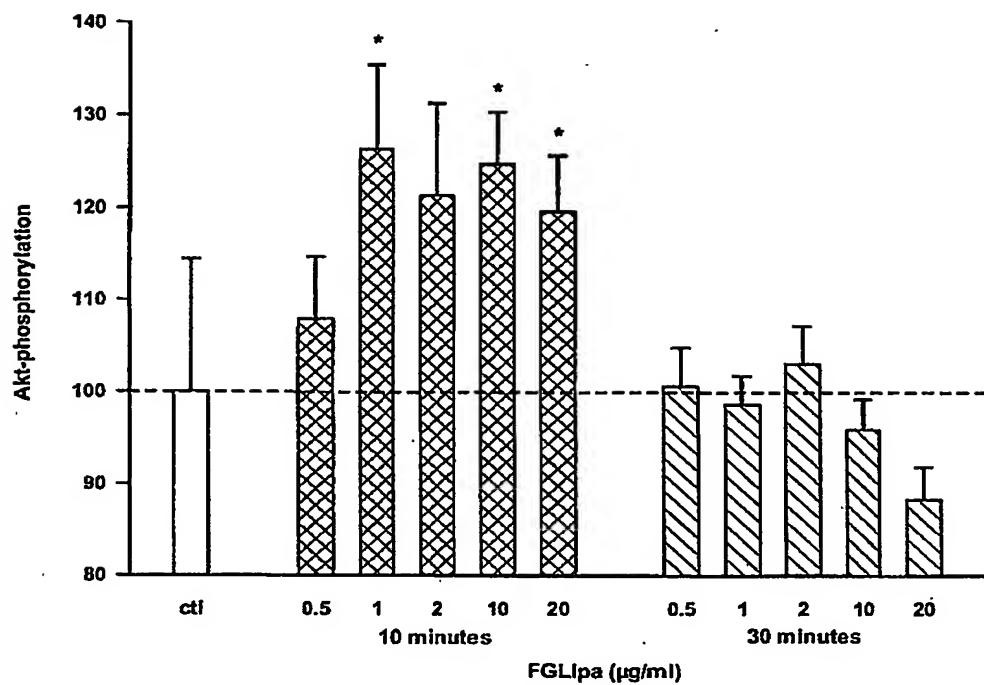


Figure 7

Modtaget

25 MAJ 2004

PVS

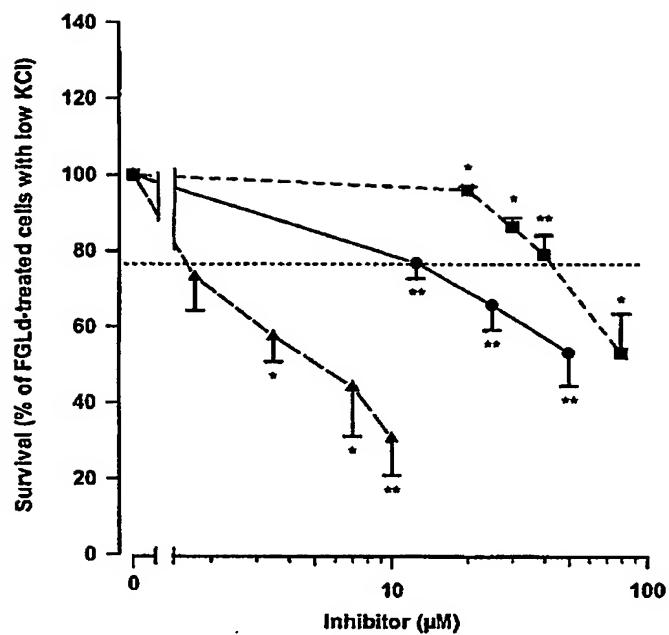


Figure 8

Modtaget
25.11.2004
PVS

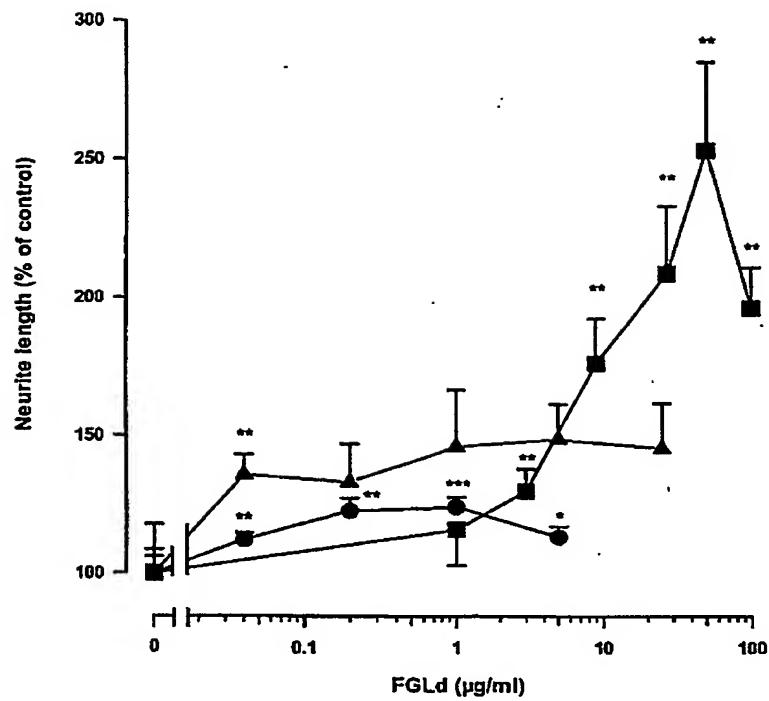


Figure 9

Modtaget
25.IV.2004
PVS

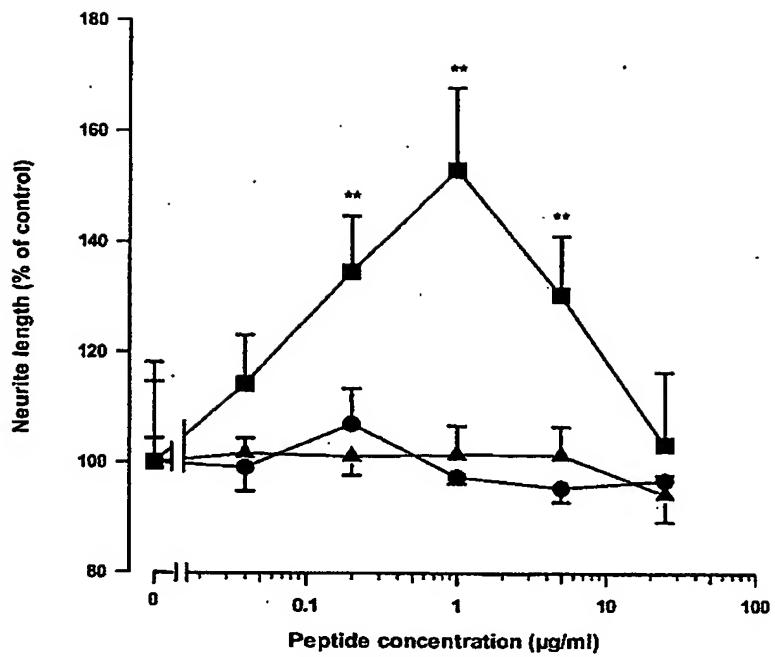


Figure 10

Modtaget
25.11.2004
PVS

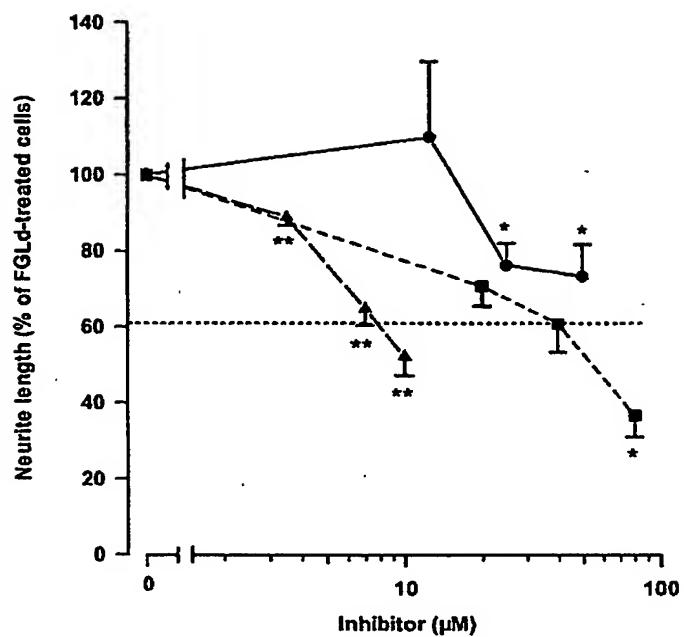


Figure 11

Modtaget

25.III.2004

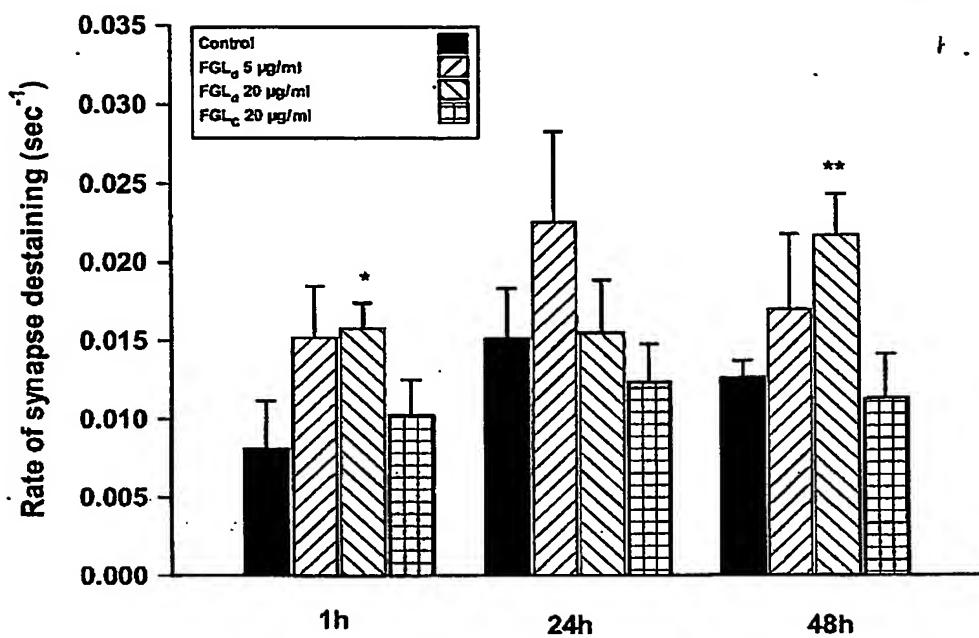


Figure 12

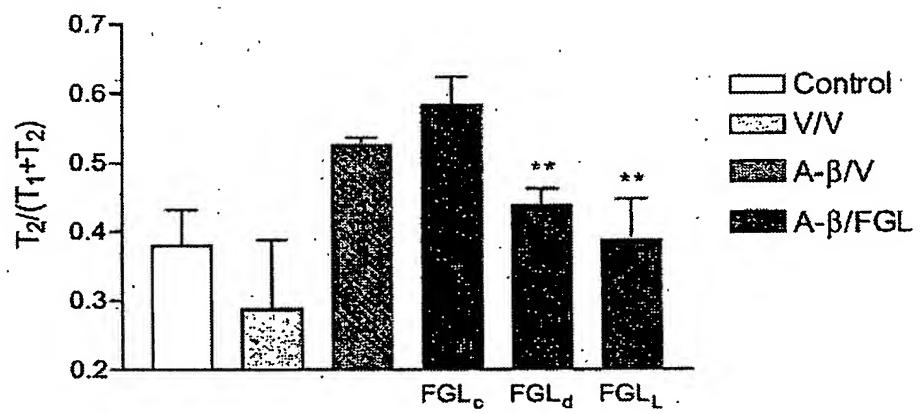


Figure 13

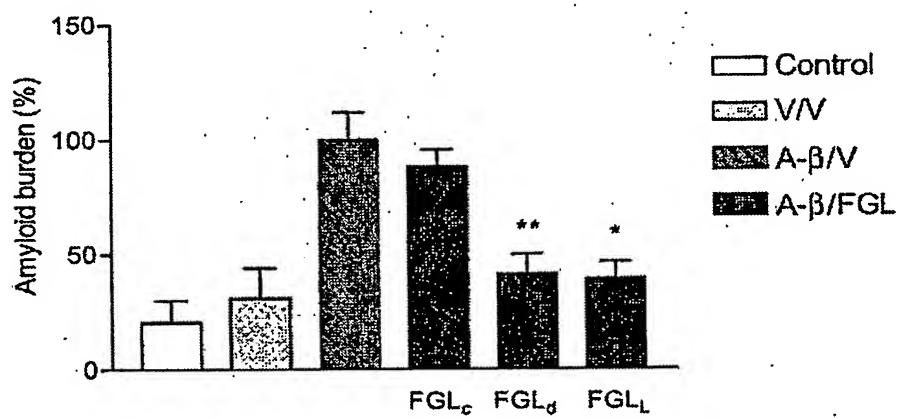


Figure 14

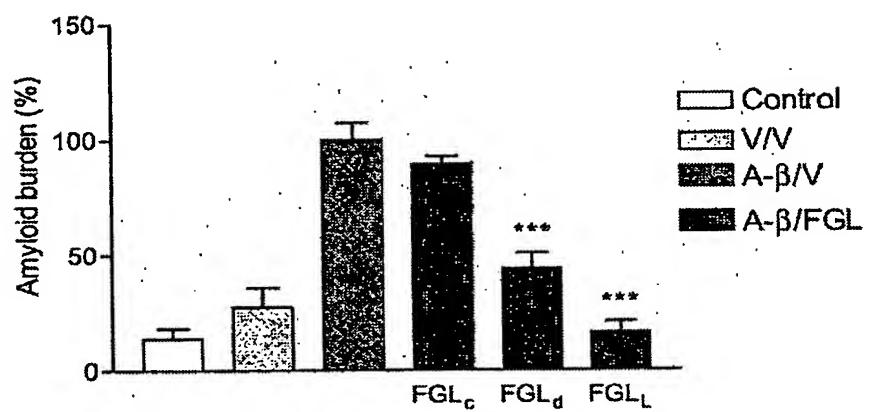


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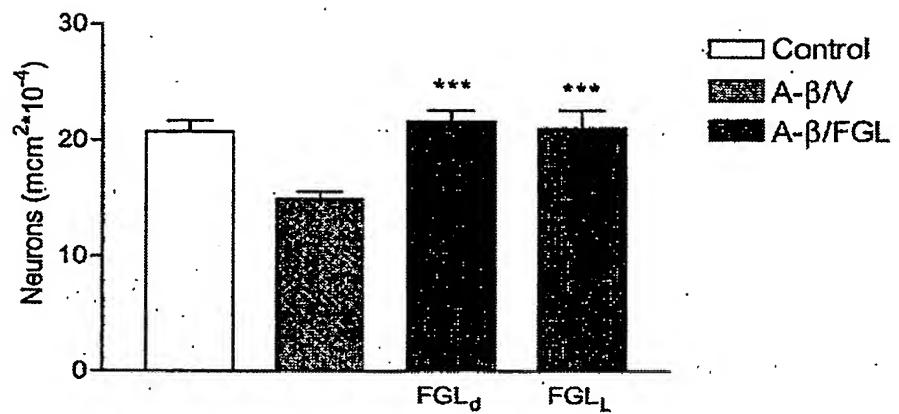


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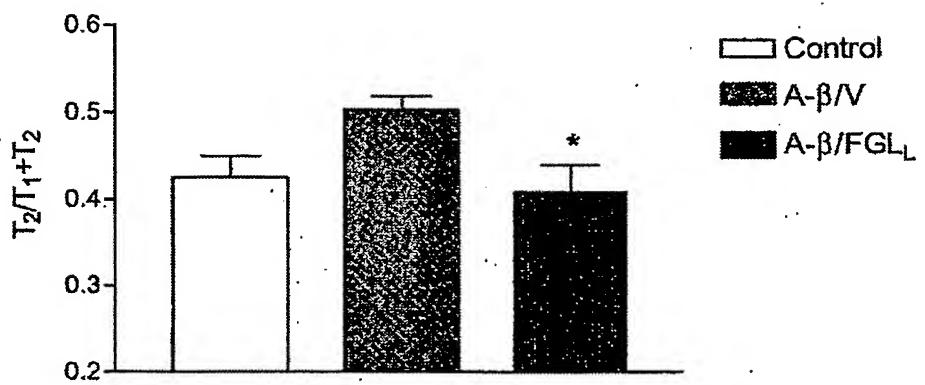


Figure 17

Amyloid burden (%)

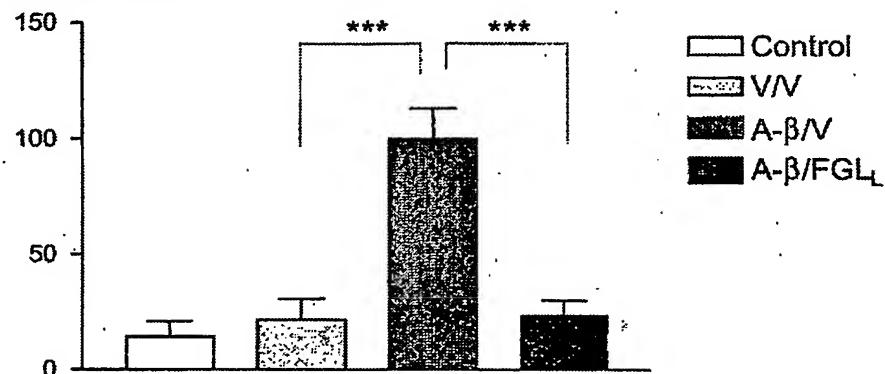


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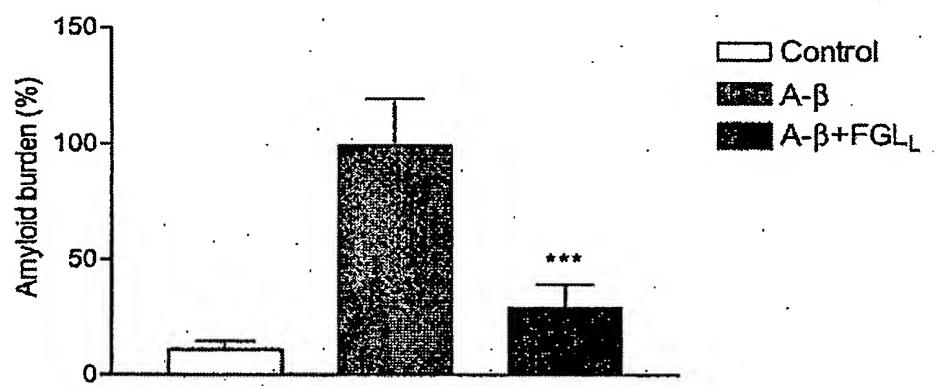


Figure 19

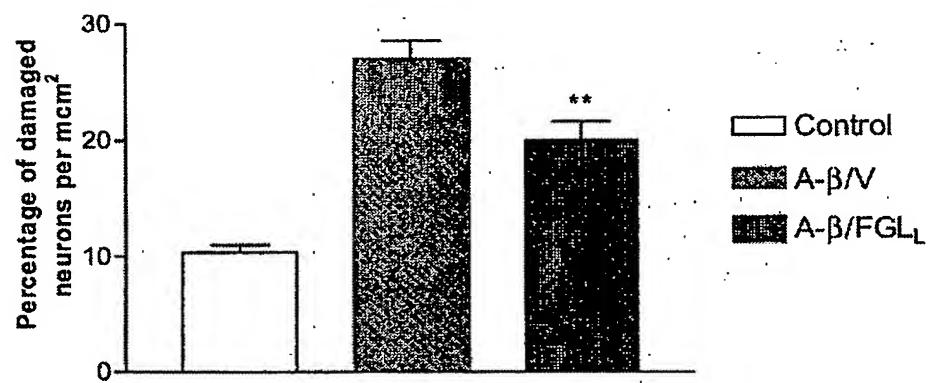


Figure 20

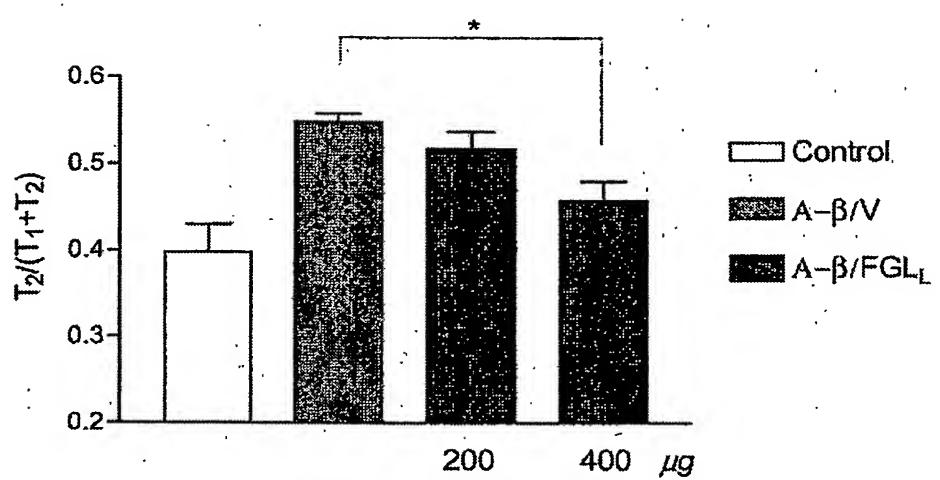


Figure 21

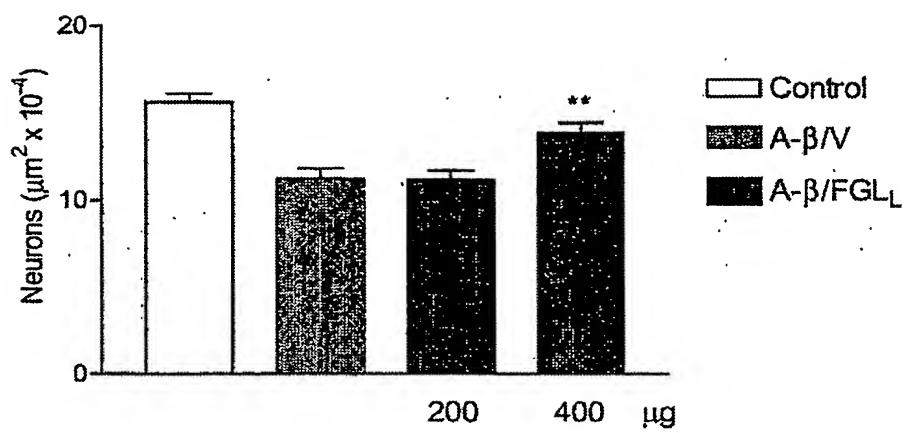


Figure 22

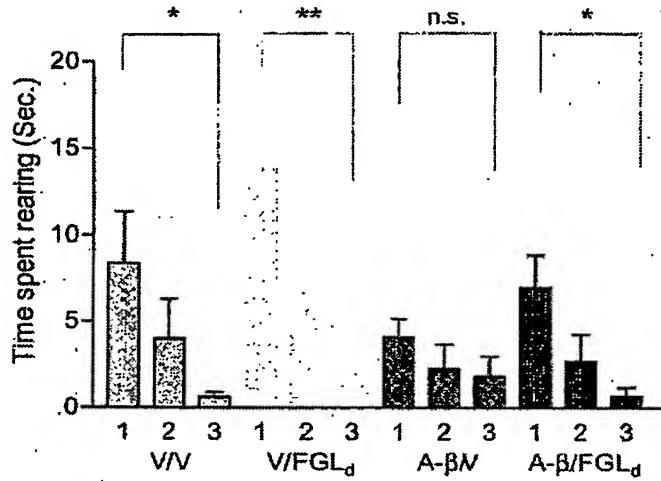


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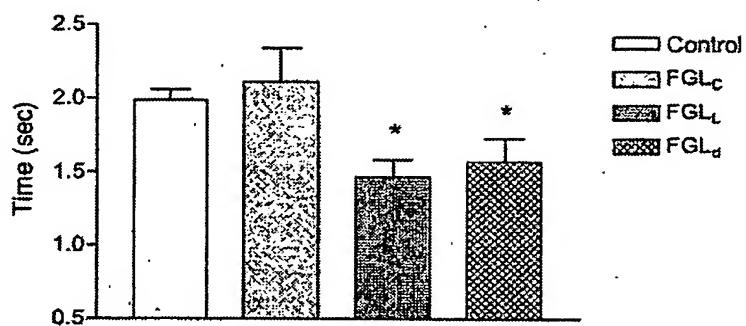


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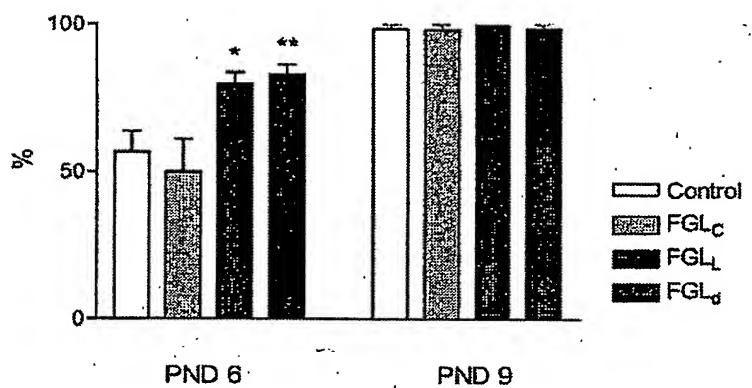


Figure 25

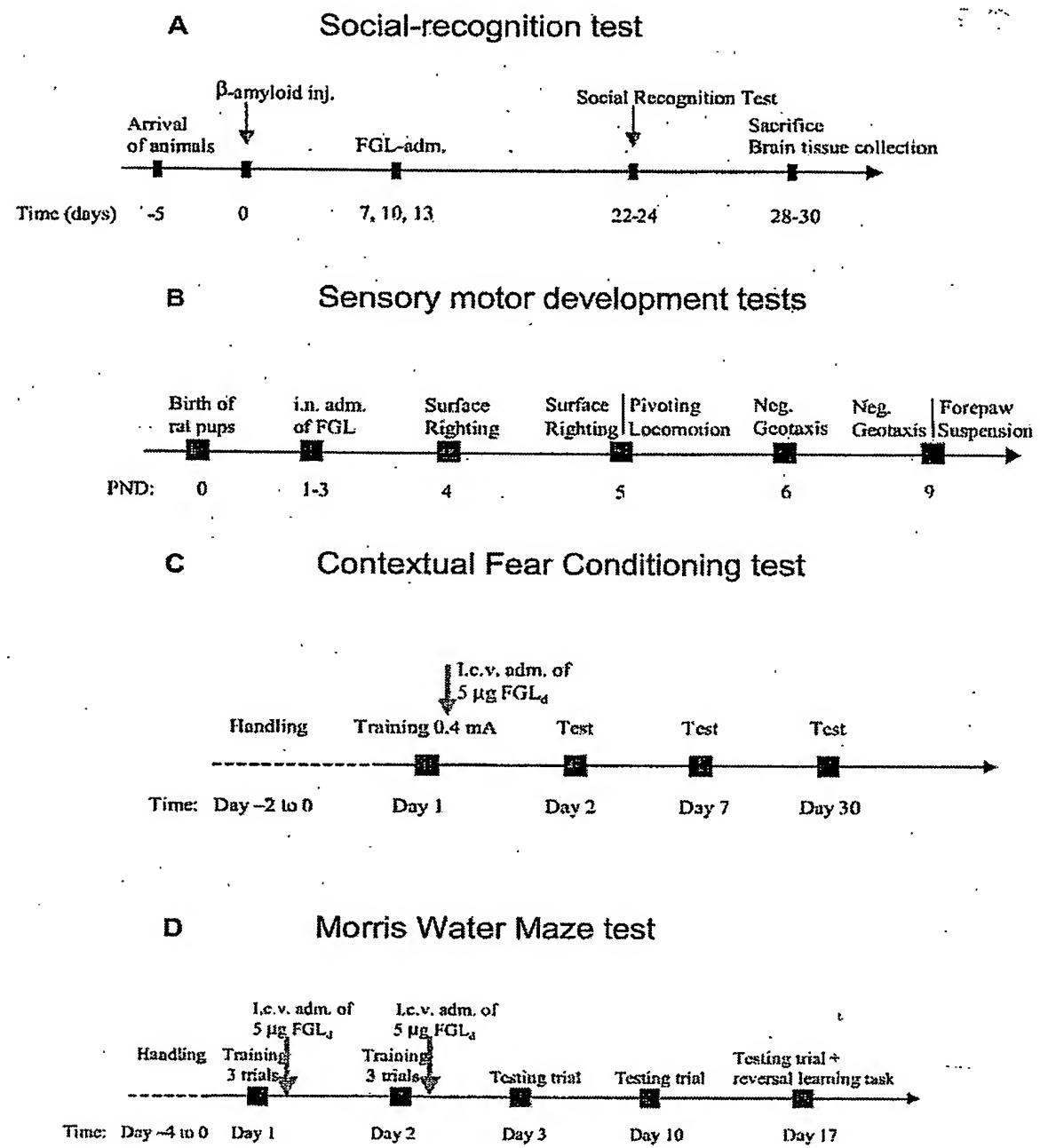


Figure 26

1
SEQUENCE LISTING

Modtaget
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<110> ENKAM Pharmaceutical A/S

<120> Compounds comprising LPA

<130> P 770 DK01

<160> 146

<170> PatentIn version 3.1

<210> 1

<211> 15

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<213> Artificial sequence

<220>

<223> NCAM Fn III, 2 [Swiss-Prot: P13591]: FGFR binding motif

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Glu Val Tyr Val Val Ala Glu Asn Gln Gln Gly Lys Ser Lys Ala
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<211> 16

<212> PRT

<213> Artificial sequence

<220>

<223> Interleukin-6 receptor beta chain [Swiss-Prot: Q00560]:
FGFR bind
ing motif

<400> 2

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<210> 3

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<212> PRT

<213> Artificial sequence

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<223> Heparan sulfate proteoglycan perlecan [Swiss-Prot: P98160]:
FGFR
binding motif

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<223> Disintegrin and metalloprotease domain 8 (ADAM-8)
[Swiss-Prot: Q05910]: FGFR binding motif

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<212> PRT

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<223> Axonal-associated cell adhesion molecule [NCBI: NP_446331]:
FGFR
binding motif

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Val Ala Glu Asn Ser Arg Gly Lys Asn Val Ala Lys Gly
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<210> 6

<211> 14

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<223> Myelin-associated glycoprotein (MAG) [Swiss-Prot: P20917]:
FGFR b
inding motif

<400> 6

Gly Glu Tyr Trp Cys Val Ala Glu Asn Gln Tyr Gly Gln Arg
1 5 10

<210> 7

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<212> PRT

<213> Artificial sequence

<220>

<223> Neuronal nicotinic acetylcholine receptor alpha 3 subunit
(CHRNA
3) [Swiss-Prot: Q8VHH6/P04757:/Q8R4G9/P32297]: FGFR binding
motif

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Lys Tyr Ile Ala Glu Asn Met Lys Ala Gln Asn Val Ala Lys Glu Ile
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<210> 8

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<212> PRT

<213> Artificial sequence

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<223> FIII,1 domain of NCAM (Swiss-Prot: P13591): FGFR binding
motif

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Thr Ile Met Gly Leu Lys Pro Glu Thr Arg Tyr Ala Val Arg
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<212> PRT

<213> Artificial sequence

<220>

<223> Granulocyte colony stimulating factor receptor precursor
(G-CSF-

5

R; CD114 antigen) [Swiss-Prot: Q99062]: FGFR binding motif

<400> 9

Lys Gly Leu Gly Glu Ile Ser Ala Ala Thr Glu Phe Lys Thr
1 5 10

<210> 10

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> NCAM Fn III, 1 [Swiss-Prot: P13591]: FGFR binding motif

<400> 10

Asn Met Gly Ile Trp Val Gln Ala Glu Asn Ala Leu Gly
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<210> 11

<211> 14

<212> PRT

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<223> FIII,1 domain of NCAM [Swiss-Prot: P13591]: FGFR binding motif

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Arg Leu Ala Ala Leu Asn Gly Lys Gly Leu Gly Glu Ile Ser
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<213> Artificial sequence

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<223> Granulocyte colony stimulating factor receptor precursor
(G-CSF-
R; CD114 antigen) [Swiss-Prot: P40223]: FGFR binding motif

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Ile Trp Val Gln Ala Glu Asn Met Leu Gly
1 5 10

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<223> Cytokine-like factor-1 precursor (CLF-1) [Swiss-Prot:
O75462]: FG
FR binding motif

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Glu Ile Trp Val Glu Ala Thr Asn Arg Leu Gly
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<223> Interleukin-23 receptor (IL-23R) [Q8NFQ9]: FGFR binding

motif

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Val Trp Val Gln Ala Ala Asn Ala Leu Gly
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<212> PRT

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<223> Complement factor 1 q , alpha polypeptide (C1QA)
[Swiss-Prot: Q9D
CM6]: FGFR binding motif

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Glu Val Trp Ile Glu Lys Asp Pro Ala Lys Gly Arg Ile
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<223> Fasciclin II precursor (FAS2) [Swiss-Prot: P22648]: FGFR
binding
motif

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Ala Thr Asn Lys Gly Gly Glu Val Lys Lys Asn Gly His Leu
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<210> 17

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<223> ADAM-19 precursor (EC 3.4.24.-) [Swiss-Prot:
Q9H013/O35674]: FGFR
binding motif

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1 5 10 15

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<223> ADAM-8 precursor (EC 3.4.24.-) [Swiss-Prot: P78325]: FGFR
binding
motif ..

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Arg Tyr Val Glu Leu Tyr Val Val Val Asp Asn Ala Glu Phe Gln
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<212> PRT

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<223> ADAM-12 precursor (EC 3.4.24.-) [Swiss-Prot: Q43184;
Q61824] : FGFR
binding motif

<400> 19

Lys Tyr Val Glu Leu Val Ile Val Ala Asp Asn Arg Glu Phe Gln Arg
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<223> Metalloproteinase-disintegrin domain containing protein
TECADAM {
AF163291] : FGFR binding motif

<400> 20

Lys Tyr Ile Glu Tyr Tyr Val Val Leu Asp Asn Gly Glu Phe Lys Lys
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<223> ADAM-33 precursor (EC 3.4.24.-) [Swiss-Prot: Q9BZ11/Q923W9] :
FGFR
binding motif

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Arg Tyr Leu Glu Leu Tyr Ile Val Ala Asp His Thr Leu Phe

1

5

10

10

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<212> PRT

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<220>

<223> ADAM-1A Fertilin alpha [Swiss-Prot: Q8R533]: FGFR binding motif

<400> 22

Lys Tyr Val Glu Met Phe Val Val Val Asn His Gln Arg Phe Gln
1 5 10 15

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<213> Artificial sequence

<220>

<223> ADAM-9 [Swiss-Prot: Q13433; Q61072]: FGFR binding motif

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Arg Tyr Val Glu Leu Phe Ile Val Val Asp Lys Glu Arg Tyr
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<210> 24

<211> 16

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<213> Artificial sequence

<220>

<223> ADAM-7 precursor [Swiss-Prot: Q9H2U9]: FGFR binding motif

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Lys	Tyr	Val	Glu	Leu	Phe	Ile	Val	Ala	Asp	Asp	Thr	Val	Tyr	Arg	Arg
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<223> ADAM-7 precursor [Swiss-Prot: O35227; Q63180]: FGFR binding motif

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Lys	Phe	Ile	Glu	Leu	Phe	Val	Val	Ala	Asp	Glu	Tyr	Val	Tyr	Arg	Arg
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<210> 26

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<223> ADAM-15 precursor [Swiss-Prot: Q9QYV0; O88839]: FGFR binding motif

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Lys	Ile	Val	Glu	Lys	Val	Ile	Val	Ala	Asp	Asn	Ser	Glu	Val	Arg	Lys
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<210> 27

<211> 14

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<220>

<223> ADAM-15 precursor [Swiss-Prot: Q13444]: FGFR binding motif

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Val Glu Leu Val Ile Val Ala Asp His Ser Glu Ala Gln Lys
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<211> 13

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<223> Neural cell adhesion protein BIG-2 precursor [Swiss-Prot: Q62845]

: FGFR binding motif

<400> 28

Val Ala Glu Asn Ser Arg Gly Lys Asn Ile Ala Lys Gly
1 5 10

<210> 29

<211> 13

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13

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<223> Neuronal glycoprotein CNTN3 [Swiss-Prot: Q07409]: FGFR
binding mo
tif

<400> 29

Ile Ala Glu Asn Ser Arg Gly Lys Asn Val Ala Arg Gly
1 5 10

<210> 30

<211> 12

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<223> NB-2 (HNB-2/NB-2), a neural cell recognition molecule of the
conta
ctin/F3 subgroup [Swiss-Prot: O94779/P97527]: FGFR binding
motif

<400> 30

Ala Glu Asn Ser Arg Gly Lys Asn Ser Phe Arg Gly
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<223> HNB-3/NB-3 [Swiss-Prot: Q9UQ52/P97528/Q9JMB8]: FGFR binding
motif

<400> 31

Ile Ala Ser Asn Leu Arg Gly Arg Asn Leu Ala Lys Gly

1 5 14 10

<210> 32

<211> 13

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<220>

<223> Putative fat-like cadherin precursor (Drosophila)
[Swiss-Prot: Q9
VW71]: FGFR binding motif

<400> 32

Ile Pro Glu Asn Ser Leu Gly Lys Thr Tyr Ala Lys Gly
1 5 10

<210> 33

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Neuronal nicotinic acetylcholine receptor alpha 3 subunit
(CHRNA3
) [Swiss-Prot: Q8VHH6/P04757/Q8R4G9/P32297]: FGFR binding
motif

<400> 33

Ile Ala Glu Asn Met Lys Ala Gln Asn Glu Ala Lys
1 5 10

<210> 34

<211> 16

<212> PRT

<213> Artificial sequence

<220>

<223> Neuronal acetylcholine receptor protein, alpha-6 chain precursor
(CHRNA6) [Swiss-prot:Q15825]: FGFR binding motif

<400> 34

Gln Phe Ile Ala Glu Asn Met Lys Ser His Asn Glu Thr Lys Glu Val
1 5 10 15

<210> 35

<211> 13

<212> PRT

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<220>

<223> ROBO-1 [O44924]: FGFR binding motif

<400> 35

Gly Glu Tyr Trp Cys Val Ala Lys Asn Arg Val Gly Gln
1 5 10

<210> 36

<211> 13

<212> PRT

<213> Artificial sequence .

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<223> ROBO-1[AF041082; Q9Y6N7]: FGFR binding motif

<400> 36

Gly Ser Tyr Thr Cys Val Ala Glu Asn Met Val Gly Lys
1 5 10

16

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<211> 14
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<220>

<223> ROBO-1 [AF041082; Q9Y6N7]: FGFR binding motif

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Gly Lys Tyr Val Cys Val Gly Thr Asn Met Val Gly Glu Arg
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<210> 38
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<212> PRT
<213> Artificial sequence

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<223> FGFR2 [Q96KM2; P21802]: FGFR binding motif

<400> 38

Asn Tyr Thr Cys Val Val Glu Asn Glu Tyr Gly
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<210> 39
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<223> FGFR2 [Q63241]: FGFR binding site

<400> 39

Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly
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<210> 40

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<223> Fc receptor-like protein 1 [Q96KM2] / fragment of IFGP 1
[Q96PJ6]:

FGFR binding motif

<400> 40

Gln Tyr Tyr Cys Val Ala Glu Asn Gly Tyr Gly
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<210> 41

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<213> Artificial sequence

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<223> Junctional adhesion molecule (JAM-1) [Q9JKD5/O88792]: FGFR
bindin
g motif

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Gly Glu Tyr Tyr Gln Glu Ala Glu Gln Asn Gly Tyr Gly
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Gly Asn Tyr Thr Cys Leu Val Glu Asn Glu Tyr Gly
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<210> 43

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<223> Contactin precursor (Neural adhesion molecule F3) [Q63198;/P1260;
Q12860]: FGFR binding motif

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Gly Met Tyr Gln Cys Leu Ala Glu Asn Ala Tyr Gly
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<211> 11

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<213> Artificial sequence

<220>

<223> Contactin precursor (Neural adhesion molecule F3/F11)

[Q28106]: F
GFR binding motif

<400> 44

Gly Met Tyr Gln Cys Ala Glu Asn Thr His Gly
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<210> 45

<211> 12

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<223> Contactin precursor (Neural adhesion molecule F3/F11)
[Q28106]: F
GFR binding motif

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Gly Ile Tyr Tyr Cys Leu Ala Ser Asn Asn Tyr Gly
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<223> IFGP2 [Q96PJ5]: FGFR binding motif

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Gly Gly Tyr Tyr Cys Thr Ala Asp Asn Ser Tyr Gly
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20

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<223> Neurofascin precursor [Q90924]: FGFR binding motif

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Gly Glu Tyr Gln Cys Phe Ala Arg Asn Asp .Tyr Gly
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<223> Neurofascin [Q90924]: FGFR binding motif

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Gly Glu Tyr Phe Cys Leu Ala Ser Asn Lys Met Gly
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<223> Neurofascin 155 Da isoform [Q91Z60]: FGFR binding motif

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Gly Glu Tyr Gln Cys Phe Ala Arg Asn Lys Phe Gly
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<223> Neurofascin 155 Da isoform [Q91Z60]: FGFR binding motif

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Gly Glu Tyr Phe Cys Leu Ala Ser Asn Lys Met Gly
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<210> 51

<211> 12

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<223> Macrophage scavenger receptor 2 (MSR2) [Q91YK7]:FGFR
binding motif
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<400> 51

Gly Gly Tyr Tyr Cys Thr Ala Asp Asn Asn Tyr Gly
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<210> 52

<211> 14

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<220>

<223> Macrophage scavenger receptor 2 (MSR2) [Q91YK7]: FGFR
binding mot
if

<400> 52

Gly Asn Tyr Ser Cys Glu Ala Glu Asn Ala Trp Gly Thr Lys
1 5 10

<210> 53

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Neural cell adhesion molecule L1 [Q9QYQ7; Q9QY38; P11627;
Q05695;
P32004]: FGFR binding motif

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Gly Glu Tyr Thr Cys Leu Ala Glu Asn Ser Leu Gly
1 5 10

<210> 54

<211> 13

<212> PRT

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<220>

<223> Neural-glial cell adhesion molecule Ng-CAM [Q03696]: FGFR
binding
motif

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Gly Glu Tyr Glu Cys Val Ala Glu Asn Gly Arg Leu Gly
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<210> 55

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<223> FGFR3 [Q95M13; AF487554; Q99052]: FGFR binding motif

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Gly Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly Arg
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<223> FGFR3 [Q95M13; Q99052]: FGFR binding motif

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Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly
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<223> Neural cell adhesion molecule 2 (NCAM2) [P36335]: FGFR binding motif

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<210> 58

<211> 12

<212> PRT

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<223> Neural cell adhesion molecule 2 (NCAM2) [P36335]: FGFR binding motif

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Glu Tyr Thr Cys Ile Ala Asn Asn Gln Ala Gly Glu
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<210> 59

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Axonin-1 (TAG-1) [Q02246; P22063; P28685]: FGFR binding motif

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Gly Met Tyr Gln Cys Val Ala Glu Asn Lys His Leu Gly
1 5 10

<210> 60

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Neural cell adhesion molecule NCAM-140 AND ncam-140
[P13595]: FGF
R binding motif

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1 5 10

<210> 61

<211> 13

<212> PRT

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<220>

<223> Neural cell adhesion molecule NCAM-140 AND ncam-140
[P13595]: FGF
R binding motif

<400> 61

Glu Tyr Val Cys Ile Ala Glu Asn Lys Ala Gly Glu Gln
1 5 10

<210> 62

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Neurotrophin receptor tyrosin kinase type 2 (NTRKT)
[Q8WXJ5] : FGFR
binding motif

<400> 62

Gly Asp Tyr Thr Leu Ile Ala Lys Asn Glu Tyr Gly Lys
1 5 10

<210> 63

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Colorectal cancer suppressor DCC [P43146] : FGFR binding
motif

<400> 63

Gly Phe Tyr Gln Cys Val Ala Glu Asn Glu Ala Gly
1 5 10

<210> 64

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Tyrosine phosphatase LAR (ptprf) [Q9EQ17; Q64604; P23468] :

FGFR b
inding motif

<400> 64

Gly Lys Tyr Glu Cys Val Ala Thr Asn Ser Ala Gly Thr Arg
1 5 10

<210> 65

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Platelet-derived growth factor receptor beta (PDGFRB)
[Q8R406; Q0
5030]: FGFR binding motif

<400> 65

Gly Glu Tyr Phe Cys Val Tyr Asn Asn Ser Leu Gly
1 5 10

<210> 66

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Intercellular adhesion molecule-5 (ICAM-5, telencephalin)
[Q8TAM9
; Q60625]: FGFR binding motif

<400> 66

Gly Glu Tyr Glu Cys Ala Ala Thr Asn Ala His Gly Arg
1 5 10

<210> 67

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> B-cell receptor CD22 precursor (Leu-14; B-lymphocyte cell adhesio
n molecule) [P20273]: FGFR binding motif

<400> 67

Gly Ala Tyr Trp Cys Gln Gly Thr Asn Ser Val Gly Lys
1 5 10

<210> 68

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> B-cell receptor CD22 precursor (Leu-14; B-lymphocyte cell adhesio
n molecule) [P20273]: FGFR binding motif

<400> 68

Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly
1 5 10

<210> 69

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> NCAM-2 [Swiss-Prot: O15394; O35136]: FGFR binding motif

<400> 69

Arg Val Ala Ala Val Asn Gly Lys Gly Gln Gly Asp Tyr Ser
1 5 10

<210> 70

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> HCF-2 (Host cell factor 2) [Swiss-Prot: Q9Y5Z7]: FGFR
binding mot
 if: FGFR binding motif

<400> 70

Arg Val Ala Ala Ile Asn Gly Cys Gly Ile Gly Pro Phe Ser
1 5 10

<210> 71

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> ICLN (Chloride channel regulator, inducer) [Swiss-Prot:
P97506; Q
 9NRD2; Q61189; P54105]: FGFR binding motif

<400> 71

Ala Val Leu Asn Gly Lys Gly Leu Gly
1 5

<210> 72

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Galectin-12 [Swiss-Prot: Q91VD1; Q9JKX2; Q9NZ03]: FGFR
binding mo
tif

<400> 72

Ala Leu Asn Gly Gln Gly Leu Gly Ala Thr Ser
1 5 10

<210> 73

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Human receptor-like protein tyrosine phosphatase leukocyte
common
antigen-related molecule (PTPRF) [Swiss-Prot: P10586]:
FGFR bind
ing motif

<400> 73

Arg Leu Ala Ala Lys Asn Arg Ala Gly Leu Gly Glu
1 5 10

<210> 74

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Natural resistance-associated macrophage protein 1(NRAMP-1,
SLC11
A1) [Swiss-Prot: O77741]: FGFR binding motif

<400> 74

Arg Leu Gly Val Val Thr Gly Lys Asp Leu Gly Glu Ile
1 5 10

<210> 75

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> NCAM2 (180 kDa isoform precursor) [Swiss-Prot: P36335]:
FGFR bin
ding motif

<400> 75

Thr Val Thr Gly Leu Lys Pro Glu Thr Ser Tyr Met Val Lys
1 5 10

<210> 76

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Nephrin [Swiss-Prot: Q925S5; Q9JIX2; Q9ET59; Q9R044;
Q9QZS7]: FGF

R binding motif

<400> 76

Thr Leu Thr Gly Leu Lys Pro Ser Thr Arg Tyr Arg Ile
1 5 10

<210> 77

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Nephrin [Swiss-Prot: O60500]: FGFR binding motif

<400> 77

Thr Leu Thr Gly Leu Gln Pro Ser Thr Arg Tyr Arg Val
1 5 10

<210> 78

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Tyrosine phosphatase LAR (PTPRF) [Swiss-Prot : Q9EQ17]:
FGFR bind
ing motif

<400> 78

Thr Leu Leu Gly Leu Lys Pro Asp Thr Thr Tyr Asp Ile Lys
1 5 10

<210> 79

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Leukocyte common antigen-related phosphatase ptp2 precursor
(LAR-
PTP2) [Swiss-Prot: Q64605]: FGFR binding motif

<400> 79

Thr Leu Gln Gly Leu Arg Pro Glu Thr Ala Tyr Glu Leu Arg
1 5 10

<210> 80

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Protein-tyrosine phosphatase, receptor-type, S precursor
(EC 3.1.
3.48) (Protein-tyrosine phosphatase sigma) (RPTP-sigma)
[Swiss-Pr
ot: Q64699]: FGFR binding motif

<400> 80

Thr Leu Arg Gly Leu Arg Pro Glu Thr Ala Tyr Glu Leu Arg
1 5 10

<210> 81

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Tyrosine-protein kinase receptor Tie-1 precursor (TIE1.)
(EC 2.7.
1.112) [Swiss-Prot: Q06805; P35590]: FGFR binding motif

<400> 81

Thr Leu Met Asn Leu Arg Pro Lys Thr Gly Tyr Ser Val Arg
1 5 10

<210> 82

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Ephrin type-A receptor 8 precursor to (EPHA8..) (EC
2.7.1.112) (Ty
rosine-protein kinase receptor EEK) (EPH-and ELK-related
kinase)
]: [Swiss-Prot: O09127; O09127; P29322]; FGFR binding motif

<400> 82

Thr Val Ser Gly Leu Lys Pro Gly Thr Arg Tyr
1 5 10

<210> 83

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Ephrin type-A receptor 3 precursor (EC 2.7.1.112)
(Tyrosine-prote
in kinase receptor ETK1) (CEK4) (EPHA3..) [tn: P29318]:
FGFR bind
ing motif

<400> 83

Thr Ile Ser Gly Leu Lys Pro Asp Thr Thr Tyr
1 5 10

<210> 84

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Protein-tyrosine phosphatase receptor-type S precursor (EC 3.1.3.
48) (Protein-tyrosine phosphatase sigma, PTPRS)
[Swiss-Prot: Q133
32]: FGFR binding motif

<400> 84

Thr Leu Gln Gly Leu Lys Pro Asp Thr Ala Tyr
1 5 10

<210> 85

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Insulin receptor [Swiss-Prot: Q9PWN6]: FGFR binding motif

<400> 85

Leu Arg Gly Leu Lys Pro Trp Thr Gln Tyr Ala Val
1 5 10

<210> 86

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Type VII collagen [Swiss-Prot: Q63870]: FGFR binding motif

<400> 86

Ile Asp Gly Leu Glu Pro Asp Thr Glu Tyr Ile Val Arg
1 5 10

<210> 87

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Insulin-like growth factor-1 receptor precursor
[Swiss-Prot: O737
98]: FGFR binding motif

<400> 87

Leu Gln Gly Leu Lys Pro Trp Thr Gln Tyr Ala Ile
1 5 10

<210> 88

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Fibronectin [Swiss-Prot: Q95KV4; Q95KV5; P07589; Q28377;

U42594;

O95609]: FGFR binding motif

<400> 88

Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Gln
1 5 10

<210> 89

<211> 10

<212> PRT

<213> Artificial sequence

<220>

<223> Insulin-like growth factor I receptor (IGF I receptor
beta-subunit
it, IGF I receptor alpha-subunit) [Swiss-Prot: Q9QVW4;
P08069; P2
4062; Q60751; P15127; P15208]: FGFR binding motif

<400> 89

Gly Leu Lys Pro Trp Thr Gln Tyr Ala Val
1 5 10

<210> 90

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Insulin receptor-related protein precursor (EC 2.7.1.112)
(IRR) (IR-related receptor) [Swiss-Prot: P14616]: FGFR binding
motif

<400> 90

Thr Leu Ala Ser Leu Lys Pro Trp Thr Gln Tyr Ala Val

1 5 10

<210> 91

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Tenascin-R (restrictin) [Swiss-Prot: Q15568; O00531]: FGFR
bindin
g motif

<400> 91

Leu Met Gly Leu Gln Pro Ala Thr Glu Tyr Ile Val
1 5 10

<210> 92

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Neogenin precursor (NEO1..) [Swiss-Prot: Q92859; P97603;
Q90610;
P97798]: FGFR binding motif

<400> 92

Lys Gly Met Gly Pro Met Ser Glu Ala Val Gln Phe Arg Thr
1 5 10

<210> 93

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Protein tyrosine phosphatase receptor type D (PTPRD,
BA175E13.1)
[Swiss-Prot: Q8WX65; Q9IAJ1; P23468; Q64487]: FGFR binding
motif

<400> 93

Thr Leu Thr Gly Leu Lys Pro Asp Thr Thr Tyr Asp Val Lys
1 5 10

<210> 94

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Protein tyrosine phosphatase receptor type D (PTPRD,
BA175E13.1)
[Swiss-Prot: Q8WX65; Q9IAJ1; P23468; Q64487]: FGFR binding
motif

<400> 94

Ile Ser Gly Leu Gln Pro Glu Thr Ser Tyr Ser Leu
1 5 10

<210> 95

<211> 14

<212> PRT

<213> Artificial sequence

<220>

40

<223> Protein-tyrosine phosphatase receptor-type F precursor (EC
3.1.3.48) (LAR protein) (Leukocyte antigen related) [Swiss-Prot:
Q64604; Q9QW67; P10586]: FGFR binding motif

<400> 95

Thr Leu Leu Gly Leu Lys Pro Asp Thr Thr Tyr Asp Ile Lys
1 5 10

<210> 96

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Protein-tyrosine phosphatase receptor-type F precursor (EC
3.1.3.48) (Leukocyte antigen related) [Swiss-Prot: Q64604;
Q9QW67; P10586]: FGFR binding motif

<400> 96

Thr Ile Ser Gly Leu Thr Pro Glu Thr Thr Tyr Ser Ile
1 5 10

<210> 97

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> CD22 [Q9R094]: FGFR binding motif

<400> 97

Gly Asn Tyr Ser Cys Leu Ala Glu Asn Arg Leu Gly Arg

1 5 41 10

<210> 98

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> FGFR-4 [Q91742]: FGFR binding motif

<400> 98

Gly Asn Tyr Thr Cys Val Val Glu Asn Arg Val Gly
1 5 10

<210> 99

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> ICAM-5 [Q8TAM9]: FGFR binding motif

<400> 99

Gly Thr Tyr His Cys Val Ala Thr Asn Ala His Gly
1 5 10

<210> 100

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> FIII, 4 domain of L1: FGFR binding motif [Swiss-Prot: Q9QY38]

<400> 100

Leu Ser His Asn Gly Val Leu Thr Gly Tyr Leu Leu Ser Tyr
1 5 10

<210> 101

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Neuron-glia cell adhesion molecule (Ng-CaM) precursor . [Gallus ga llus]; [Swiss-Prot: Q90933]: FGFR binding motif

<400> 101

Asn Gly Val Leu Thr Gly Tyr Val Leu Arg Tyr
1 5 10

<210> 102

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Neurofascin precursor . [Gallus gallus]; [Swiss-Prot: O42414]: FGF R binding motif

<400> 102

Asn Gly Val Leu Thr Gly Tyr Asn Leu Arg Tyr
1 5 10

<210> 103

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> (CALL) Neural cell adhesion molecule. [Homo sapiens] . [
Swiss-Pro
t: O00533]: FGFR binding motif

<400> 103

Asn Gly Asn Leu Thr Gly Tyr Leu Leu Gln Tyr
1 5 10

<210> 104

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> f Neuroglian. [Manduca sexta] . [Swiss-Prot: P91767]: FGFR
bindin
g motif

<400> 104

Val Asp Glu Asn Gly Val Leu Thr Gly Tyr Lys Ile Tyr Tyr
1 5 10

<210> 105

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Protein-tyrosine phosphatase sigma [Swiss-Prot: Q75870];
and [Swiss-Prot: Q13332] [Homo sapiens] :FGFR binding motif

<400> 105

Thr His Asn Gly Ala Leu Val Gly Tyr Ser Val Arg Tyr
1 5 10

<210> 106

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> NR-CaM 12 [Rattus sp] , [Swiss-Prot: Q9QVN3]: FGFR binding motif

<400> 106

Asn Gly Ile Leu Thr Glu Tyr Ile Leu Lys Tyr
1 5 10

<210> 107

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Neurofascin 155 kDa isoform. [Rattus norvegicus], [Swiss-Prot: Q91Z60]: FGFR binding motif

<400> 107

Asn Gly Ile Leu Ile Gly Tyr Thr Leu Arg Tyr
1 5 10

<210> 108

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Neogenin (Fragment). [Gallus gallus], [Swiss-Prot: Q90610]:
FGFR b
inding motif

<400> 108

Thr His Ser Gly Gln Ile Thr Gly Tyr Lys Ile Arg Tyr
1 5 10

<210> 109

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Neogenin (Fragment). [Gallus gallus], [Swiss-Prot:
Q90610]:FGFR bi
inding motif

<400> 109

Asn Gly Lys Ile Thr Gly Tyr Ile Ile Tyr Tyr
1 5 10

<210> 110

<211> 10

<212> PRT

<213> Artificial sequence

<220>

<223> Metalloprotease 1 (pitrilysin family). [Homo sapiens] [
Swiss-Pro
t: Q9BSI6]:FGFR binding motif

<400> 110

Leu Ser His Asn Gly Ile Phe Thr Leu Tyr
1 5 10

<210> 111

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> HBRAVO/Nr-CaM. [Homo sapiens]. [Swiss-Prot: Q92823; O15179]:
FGFR b
inding motif

<400> 111

Asn Gly Ile Leu Thr Glu Tyr Thr Leu Lys Tyr
1 5 10

<210> 112

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Protein-tyrosine phosphatase kappa precursor (EC 3.1.3.48)

(R-PTP
-kappa). [Homo sapiens]. [Swiss-Prot: Q15262]: FGFR binding motif

<400> 112

Leu Asp Pro Asn Gly Ile Ile Thr Gln Tyr Glu Ile Ser Tyr
1 5 10

<210> 113

<211> 11

<212> PRT

<213> Artificial sequence

<220>

<223> Neogenin precursor (NEO1..). [Homo sapiens and Mus
musculus] [Swiss
-Prot: Q92859; P97798]: FGFR binding motif

<400> 113

Asn Gly Lys Ile Thr Gly Tyr Ile Ile Tyr Tyr
1 5 10

<210> 114

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> Neural cell adhesion L1(SPLICE ISOFORM 2)[Homo sapiens
[Swiss-P
rot: P32004]; [Mus musculus Swiss-Prot: Q9QY38]: FGFR
binding
motif

<400> 114

His Leu Glu Val Gln Ala Phe Asn Gly Arg Gly Ser Gly Pro Ala

1 5 48 10 15

<210> 115

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> NB-2. [Rattus norvegicus] [Swiss-Prot: P97527]:FGFR binding motif

<400> 115

His Leu Thr Val Arg Ala Tyr Asn Gly Ala Gly Tyr Gly Pro
1 5 10

<210> 116

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> Neural cell adhesion protein BIG-2 precursor. [Rattus norvegicus]
Swiss-Prot: Q62845]: FGFR binding motif

<400> 116

His Leu Ser Val Lys Ala Tyr Asn Ser Ala Gly Thr Gly Pro Ser
1 5 10 15

<210> 117

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> Axonal-associated cell adhesion molecule.[Homo sapiens].
[Swiss-P
rot: Q8TC35]:FGFR binding motif

<400> 117

His Leu Ala Val Lys Ala Tyr Asn Ser Ala Gly Thr Gly Pro Ser
1 5 10 15

<210> 118

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Contactin A/F3/F11.[Xenopus laevis] [Swiss-Prot: O93250]:
FGFR bi
nding motif

<400> 118

Asn Leu Glu Val Arg Ala Phe Asn Ser Ala Gly Asp Gly Pro
1 5 10

<210> 119

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Neural cell adhesion molecule CALL.[Homo
sapiens][Swiss-Prot: O0
0533]:FGFR binding motif

50

<400> 119

His Leu Thr Val Leu Ala Tyr Asn Ser Lys Gly Ala Gly Pro
1 5 10

<210> 120

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Neuron-glia cell adhesion molecule (Ng-CaM)
precursor. [Gallus gal-
lus] [Swiss-Prot: Q909339]: FGFR binding motif

<400> 120

Leu Arg Val Leu Val Phe Asn Gly Arg Gly Asp Gly Pro
1 5 10

<210> 121

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Contactin precursor (Neural cell recognition molecule F11). [Gallu-
s gallus] [Swiss-Prot: P14781]: FGFR binding motif

<400> 121

His Ile Asp Val Ser Ala Phe Asn Ser Ala Gly Tyr Gly Pro
1 5 10

<210> 122

<211> 10

<212> PRT

<213> Artificial sequence

<220>

<223> SLIT [Drosophila melanogaster] [Swiss-Prot: Q9XYV4]: FGFR
binding motif

<400> 122

His Leu Ala Val Glu Leu Phe Asn Gly Arg
1 5 10

<210> 123

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Galectin-4. [Mus musculus] [Swiss-Prot: Q8K419, P38552]:
FGFR bin ding motif

<400> 123

Leu Glu Leu Gln Ser Ile Asn Phe Leu Gly Gly Gln Pro Ala
1 5 10

<210> 124

<211> 14

<212> PRT

<213> Artificial sequence

<220>

52

<223> HNB-2. [Homo sapiens] Swiss-Prot: O94779: FGFR binding motif

<400> 124

His Phe Thr Val Arg Ala Tyr Asn Gly Ala Gly Tyr Gly Pro
1 5 10

<210> 125

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> The EFL peptide (from the FIII,3 domain of L1) [Swiss-Prot:
P3200 4]: FGFR binding motif

<400> 125

His Leu Glu Val Gln Ala Phe Asn Gly Arg Gly Ser Gln Pro Ala
1 5 10 15

<210> 126

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Fragment of Neuroglian (Drosophila) [Swiss-prot: P202419]:
FGFR b
inding motif

<400> 126

Val Ile Ala Asp Gln Pro Thr Phe Val Lys Tyr Leu Ile Lys
1 5 10

<210> 127

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Fragment of Fibronectin (bovine) [Swiss-prot: P07589]: FGFR
bindi
ng motif

<400> 127

Thr Ile Lys Gly Leu Arg Pro Gly Val Val Tyr Glu Gly Gln
1 5 10

<210> 128

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Tenascin (chick) [Swiss-prot: P10039]: FGFR binding motif

<400> 128

Thr Leu Thr Glu Leu Ser Pro Ser Thr Gln Tyr Thr Val Lys
1 5 10

<210> 129

<211> 14

<212> PRT

<213> Artificial sequence

<220>

54

<223> Ephrin type A receptor2 [Swiss-prot: Q8N3Z2]: FGFR binding motif

<400> 129

Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln
1 5 10

<210> 130

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> LAR [Swiss-prot Q9VIS8]: FGFR binding motif

<400> 130

Thr Val Ser Asp Val Thr Pro His Ala Ile Tyr Thr Val Arg
1 5 10

<210> 131

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> RTK (Tie-1,hu) [Swiss-prot P35590]: FGFR binding motif

<400> 131

Ile Ile Arg Gly Leu Asn Ala Ser Thr Arg Tyr Leu Phe Arg
1 5 10

<210> 132

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> RTK (Tie-1, hu) [Swiss-prot P35590]: FGFR binding motif

<400> 132

Thr Leu Met Asn Leu Arg Pro Lys Thr Gly Tyr Ser Val Arg
1 5 10

<210> 133

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Consensus sequence (conserved domain database) : FGFR
binding mot
if

<400> 133

Thr Leu Thr Gly Leu Lys Pro Gly Thr Glu Tyr Glu Val Arg
1 5 10

<210> 134

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> The beta-common cytokine receptor of IL-3. IL-5 and GmCsf
[Swiss-
prot P32927]: FGFR binding motif

<400> 134

Gly Pro Glu His Leu Met Pro Ser Ser Thr Tyr Val Ala Arg
1 5 10

<210> 135

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Unc-22 (C. Elegance) [Swiss-prot: Q23550]: FGFR binding motif

<400> 135

Arg Val Thr Gly Leu Thr Pro Lys Lys Thr Tyr Glu Phe Arg
1 5 10

<210> 136

<211> 14

<212> PRT

<213> Artificial sequence

<220>

<223> Consensus sequence (conserved domain database): FGFR binding motif

<400> 136

Thr Leu Thr Gly Leu Lys Pro Gly Thr Glu Tyr Glu Phe Arg
1 5 10

<210> 137

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> Consensus sequence (conserved domain database): FGFR binding motif

<400> 137

Glu Val Arg Val Gln Ala Val Asn Gly Gly Gly Asn Gly Pro Pro
1 5 10 15

<210> 138

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Drosophila Neuroglian [Swiss-prot: P20241]: FGFR binding motif

<400> 138

Leu Ile Lys Val Val Ala Ile Asn Asp Arg Gly Glu
1 5 10

<210> 139

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Fibronectin (mouse) [Swiss-prot: P11276]: FGFR binding motif

<400> 139

Val Val Ser Ile Ile Ala Val Asn Gly Arg Glu Glu
1 5 10

<210> 140

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Fibronectin (bovine) [Swiss-prot: P07589]: FGFR binding motif

<400> 140

Val Val Ser Val Tyr Ala Gln Asn Gln Asn Gly Glu
1 5 10

<210> 141

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Tenascine (chick) [Swiss-prot: Q90995]: FGFR binding motif

<400> 141

Thr Ile Ser Leu Val Ala Glu Lys Gly Arg His Lys
1 5 10

<210> 142

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> L1 (human, F3,EFL) [Swiss-prot: P32004]: FGFR binding motif

<400> 142

His Leu Glu Val Gln Ala Phe Asn Gly Arg Gly Ser Gly Pro Ala
1 5 10 15

<210> 143

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> L1 (mouse, F3,EFL) [Swiss-prot: P11627]: FGFR binding motif

<400> 143

His Val Glu Val Gln Ala Phe Asn Gly Arg Gly Leu Gly Pro Ala
1 5 10 15

<210> 144

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> L1 (rat, F3,EFL) [Swiss-prot: Q05695]: FGFR binding motif

<400> 144

His Val Glu Val Gln Ala Phe Asn Gly Arg Gly Leu Gly Pro Ala
1 5 10 15

<210> 145

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Consensus sequence (conserved domain database): FGFR
binding motif
f

<400> 145

Glu Phe Arg Val Arg Ala Val Asn Gly Ala Gly Glu Gly
1 5 10

<210> 146

<211> 15

<212> PRT

<213> Artificial sequence

<220>

<223> The beta-common cytokine receptor of IL-3, IL-5 and GmCsf
[Swiss-
prot: P32927]: FGFR binding motif

<400> 146

Val Ala Arg Val Arg Thr Arg Leu Ala Pro Gly Ser Arg Leu Ser
1 5 10 15